

AMERICAN JOURNAL OF BOTANY

VOL. X

FEBRUARY, 1923

No. 2

ALTERNATION OF SEXES AND INTERMITTENT PRODUCTION OF FRUIT IN THE SPIDER FLOWER (*CLEOME SPINOSA*)¹

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(Received for publication April 6, 1922)

Irregularities in the formation of reproductive organs, such as are seen in the phenomena of intersexualism in both plants and animals, have two points of special interest. First, they involve a particular type of sterility of various grades and degrees of expression, which in plants often affects the production of fruit and seeds and becomes a matter of practical importance in respect to crop production and in the breeding of various economic plants. A second point of interest is in the bearing which the phenomena of intersexualism have on questions of sex differentiation, the alternation of sex, and the evolutionary tendencies in reproduction.

In its general significance, several points regarding sterility from intersexualism are clear. In plants it tends to the alternative development of one or the other kind of sex organs, giving, in comparison to the fundamental condition of hermaphroditism, a one-sided sterility. There is incomplete development or abortion of one or the other of the sex organs which is discriminating and which results in alternative development, with, however, many grades in the relative development. Thus, in plants, the so-called "sterile" intersexes are, in general, individuals that are predominantly male and often highly functional as such. These individuals are sterile only in the sense that they are fruitless. Also the so-called "self-sterile" individuals and varieties of plants, as is well shown in the cultivated grapes in which sterility from intersexualism is well marked, are predominantly female and able to function feebly or not at all as males. They are productive of fruit only when properly pollinated from male or hermaphroditic individuals. Very seldom, if ever, is complete sexual impotence for a plant as a whole seen as a condition of intersexualism, as is frequently the case in sterility from hybridity.

But in many cases of intersexualism in animals, to which attention has recently been especially directed, the complete sterility of individuals is very frequent. Here, however, the condition arises in dioecious forms and involves the partial change of an organ from one sex to the other after

¹Contribution from the New York Botanical Garden, No. 239.

[The *Journal* for January (10 : 1-56) was issued February 3, 1923].

differentiation has been partly achieved. This often results in a more or less complete sexual impotence or sterilization, a condition which has naturally been very generally regarded as abnormal and pathological. This is, however, not the case in those species of plants which are prevailing dioecious or monoecious, for here, as well shown in the muskmelons, there is a tendency to produce flowers that are fully functional as hermaphrodites.

Whether, however, intersexualism results in complete sterility, as it frequently does in dioecious animals, or in one-sided sterility, as is the rule in hermaphroditic plants, the physiological basis for these variations in sex is to be regarded as most fundamental in the determination and expression of sex.

It is, furthermore, to be recognized that the mixture of sexes, with blending and changes in the character of the organs, often results in a wide range of variation in the morphological character of the different sex organs produced by a single individual. In many plants, the flowers on a single individual may be staminate, pistillate, and hermaphroditic, with also many intergrading types, thus exhibiting many grades of sexual impotence with marked differences in the ability to produce fruit.

These cases of partial variability in sex are of special interest, for here the various conditions of alternative impotence with corresponding irregularities in the production of fruit are all seen among the flowers of a single individual. In such cases there is also opportunity to observe whether the variations are irregular and sporadic or whether they are related to a definite period in development or are otherwise periodic. It is with special reference to these questions that the changes in the character of the flowers of *Cleome spinosa* L. are here reported as decidedly alternative and repeatedly cyclic, resulting in the intermittent production of fruit.

OBSERVATIONS ON *CLEOME SPINOSA*

This species is most favorable material for a study of variation in the sex of flowers in relation to the development of the plant as a whole. It has long been known as having mixed flowers, yet the species has not become dioecious. All the individuals of the species are apparently quite alike in respect to the general range of variations in the sex of the flowers. The species is a quick-growing herbaceous annual. The first flowers open on the main raceme when the plant is relatively small—about two feet tall—and while the lateral branches are scarcely visible. The main raceme continues to elongate, producing flowers daily, often for a period of from eight to twelve weeks. Meanwhile a dozen or more lateral branches develop, and these may in turn branch. All the branches grow rapidly and produce flowers in abundance. When autumn arrives, well-grown plants are five or more feet tall with a spread of branches of as many feet in diameter. There has been a long period of bloom, often covering as many as ninety days, and this has been for the most part coincident with the period of

rapid vegetative development. By far the greater amount of the vegetative growth of the plant takes place after the blooming begins. The first fruits are ripe and shed their seeds when the plant is only about half grown. The period of vegetative vigor overlaps that of the flowering and reproductive vigor in a decided degree and to an extent seldom seen in plants. Only during the last few days of bloom do vegetative growth and vigor noticeably wane.

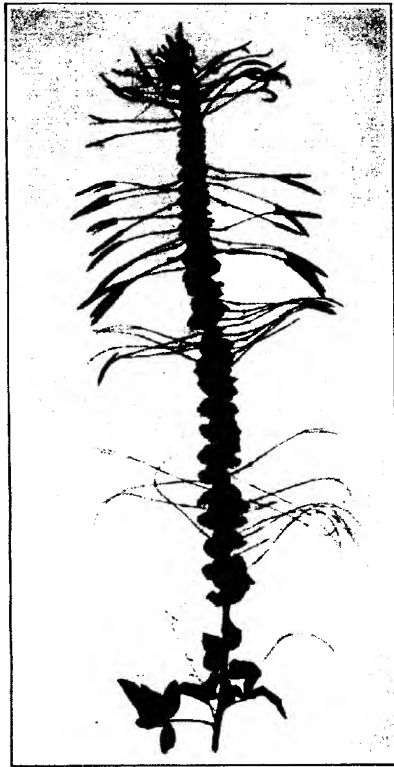


FIG. 1. Main raceme of a plant of *Cleome spinosa* at the close of the blooming period, showing the intermittent production of fruit. This raceme was about $3\frac{1}{2}$ feet long. The pods on the lower half have dehisced.

At the close of the growth of the plant, about September 15th to October 5th as grown at the New York Botanical Garden, the main branch of a plant from the point where the flowers are first produced appears as shown

in figure 1, the raceme being about three feet in length. Lateral branches are similar but frequently somewhat shorter. It is to be noted that the pods are in groups separated by sections of the stem upon which no fruit was formed. Fruit formation is therefore decidedly *intermittent*.

This habit of producing fruit intermittently was observed by the writer in groups of plants grown in ornamental planting in the Botanical Garden during previous years. For the purpose of making special observations on the conditions involved in the intermittent production of fruit, a crop of 128 plants was grown in 1921. These plants were examined frequently throughout the entire period of bloom, and records were taken for each individual plant as to the character of the flowers opening at a particular time. At the end of the season, observations on the distribution of fruit in regard to the record for the flowers was made. Controlled self- and cross-pollinations were made on many plants.

Every one of the 128 plants produced many pods and the seeds were numerous, but without exception there was decided intermittency in the production of fruit. On several plants there was considerable irregularity in the distribution of pods, but for most plants the pods were in several groups quite as shown in figure 1.

The study of the flowers from day to day together with the results of controlled pollination showed that the intermittent production of fruit is due to repeated cyclic changes in the morphological character of the flowers, which in the course of the cycles give many grades of intersexes. The flowers of any individual plant varied from perfect or fully hermaphroditic flowers to flowers that were functional only as males or only as females, with also innumerable intergrades as to the relative abortion of pistils or stamens. As a rule, however, the loss of sex is decidedly *one-sided*. When the flowers are hermaphroditic or are female, fruit is produced provided pollination is accomplished; when the flowers are male only, no fruit is produced. The plants pass through alternating periods when the flowers are predominantly hermaphroditic or are female, during which they are productive of fruit, to periods when the flowers are predominantly or only male and fruitless.

The sex character of the flowers, therefore, varies in cycles, which variation makes the intermittent production of fruit a necessary result. The main raceme shown in figure 1 bloomed for a period of 107 days, and on it were produced about 250 flowers. During this time there were for this particular raceme five periods when hermaphroditic and female flowers were produced, with intervening periods when the flowers were staminate only.

In selecting material to illustrate these changes in sex, flowers opening on the same raceme at the same time were taken, the selection being made at a time when the variation was marked. It is, however, seldom that the variation on any one date represents the complete range observed for a plant during a complete cycle. As is shown in the figures, the sex organs

(pistils and stamens) when fully developed are large and conspicuous, and it is easy to observe variations in the degree of their development. The petals were removed from the flowers shown in Plate VI.

The three flowers shown in figure 2, Plate VI, were situated in a raceme in the succession shown and illustrate the range seen for the plant on the particular date when the photo was made; all the flowers were fully and very uniformly male; but the pistils were either normal and functional as in *a*, decidedly aborted and functionless as in *c*, or less conspicuously aborted as in *b*. On the particular date the flowers were varying in respect to femaleness. On other dates, however, maleness was quite as variable.

Maleness is well developed in all the flowers shown in figure 3, although the filaments vary in length and there is one stamen fully aborted in *b* and one in *c*. The pistils vary from the highly developed and functional as at *a* to the extremely aborted as in *d*. Figure 4 represents flowers of the same plant a few days later, showing extreme abortion in stamens of some flowers and some variation in the size of pistils, although all pistils were functional.

The pistils of the various flowers shown in figure 5 are either well developed (*a*, *b*, and *c*), or decidedly aborted (*d*, *e*, and *f*). The development of stamens is very irregular, and the extremes are seen for a single flower in the various grades as to length of filament and size, development, and dehiscence of anthers. Such irregularities as these are frequently seen, and for numerous plants the condition was more or less present throughout the period of bloom, with, however, no pod production for the flowers that had only aborted pistils.

Figure 6 shows two typical flowers of a plant on a date when the flowers could function only as females.

The four flowers shown in figure 7 show grades of abortion in both pistils and stamens and illustrate very well how the abortion tends to be one-sided, affecting first stamens and then pistils.

The many controlled pollinations that were made revealed that there was in these plants no limitation to fertility through physiological incompatibilities in fertilization. Every plant was highly productive of seed whenever pollen of dehiscing stamens was used on well-developed pistils either in self- or in cross-pollinations. Rudimentary pistils always failed to set seed. Pistils over 2 cm. in length usually produced seed.

Examination of pollen and tests for germination were made of pollen from all sorts of anthers. In large, well-developed anthers, 95% or more of the pollen grains appeared to be normal, and on a sugar-agar medium as many as 80% often germinated, producing tubes as long as 750 μ . In such rudimentary stamens as at *d*, figure 4, only a few shriveled, empty, partly developed pollen grains were present which did not even swell up when placed in water. In the large-sized but indehiscent anthers of short stamens as in *c*, figure 4, varying percentages of the pollen appeared to be normal, but in no case did the pollen of such indehiscent anthers germinate when

removed and placed on the same medium which gave good germination for the pollen of normally dehiscent stamens. There were many anthers that were partly dehiscent, that is, they opened to some extent, and the pollen thus shed was often viable in tests and productive of seed in controlled pollinations.

As a rule, the loss of sex for individual flowers was one-sided. When the pistil was rudimentary the stamens tended to be well developed as at *c*, figure 2. When the stamens were aborted the pistil was usually of good size as at *c*, figure 4. Occasionally, however, the pistil in flowers with aborted stamens was undersized as at *d*, figure 4, but cases of complete or extreme abortion of all stamens and of the pistil in the same flower were not observed.

The entire lot of plants were grown throughout under very uniform conditions which favored continued vegetative vigor, and only one generation has been critically studied. Development under conditions which affect differently the vegetative vigor and the length of the growing period may influence and possibly decidedly change the behavior in respect to cyclic changes, quite as such conditions are known to affect the sex of certain plants, particularly of *Arisaema triphyllum* (Pickett, 1915; Schaffner, 1922), from year to year. Definite evidence regarding the direct or indirect influences of environment and the somewhat synchronous changes of sex in the spider flower remains to be obtained.

At the close of the season, when the vigor of plants perceptibly wanes, all parts of the flower, corolla, pistils, and stamens alike, are uniformly undersized. Whether the last flowers that are produced on a plant that reaches old age before being killed by freezing temperatures are as a rule predominantly pistillate or staminate was not determined with certainty. On many such plants the last flowers were decidedly weak in maleness, but for other plants such flowers were decidedly male or bisexual.

SUMMARY

In the cultures of *Cleome spinosa* grown for this observational study there was wide variation in the morphological character of the flowers in regard to the relative development of the two kinds of sex organs. The entire range of variations was seen among the flowers of a single plant, giving bisexual flowers, flowers that were functional only as males or as females, and many intergrading types. The variation from one extreme to the other was repeatedly cyclic, which condition results in the intermittent production of fruit.

All of the 128 plants grown under special observation were quite similar; all exhibited the extreme ranges of flower forms or intersexes; in all the production of fruit was more or less intermittent; none was exclusively staminate or pistillate.

DISCUSSION

The alternative loss of maleness and femaleness in the flowers of *Cleome spinosa* and the recurring periodic changes in the sex of the flowers are to be regarded as phenomena of internal and biogenetic regulation closely related to those influences which determine the development of the plant as a whole.

It is, of course, well recognized that in plants as contrasted with animals there is continually the formation of really *new* organs from a persistent embryonic complex of cells and that this continues until the maturity and death of the plant as a whole. Internal and biogenetic relations of correlation and self-regulation, operating independently or in response to external influences, are hence repeated successively in determining the character of the new organs in the same fashion as they operate once for all in the animal. When there is in addition a long flowering period which overlaps and is coincident with the period of the most vigorous vegetative development, as is the condition in this robust annual *Cleome spinosa*, the conditions are most favorable for a study of the factors influencing the differentiation of sex.

The fact that the loss of sex organs in the flowers of the spider flower is very decidedly one-sided and qualitative is of special significance. When the stamens are aborted the pistil is as a rule functional, and in many cases it is well developed; when the pistil is aborted the stamens are often highly developed and functional. Here, as is the rule in plants, intersexualism does not lead to sterility of the plant or of a flower as a whole. Not a flower was found in which the pistil and all the stamens were extremely aborted, and rather rarely was the abortion of one sex accompanied by the decided abortion of the other sex in the same flower. Abortion of pistils was frequently accompanied by irregular abortion among the various stamens of a flower, but the same irregularity in maleness was also seen for flowers in which there was no abortion of the pistil (see the flowers of fig. 4). While the expression of sex in at least half of the flowers of a plant is decidedly one-sided and alternative, it is not mutually exclusive, for on every plant many bisexual flowers are produced.

It should be noted that the influences operate primarily and almost discriminatingly on the organs of sex. The pedicels, sepals, and petals are often uniformly well developed for all the types of flowers; but undersized flowers were to be seen (*c* of fig. 3, and *d* of fig. 4) in which the flower as a whole is undersized. Such cases, if more general, would suggest a direct relation to waning vigor and decreased food supply such as may be considered to be the direct cause of undersized flowers and of loss of sex in gynomonocious forms at the end of a period of bloom. That the conditions are more intricate in *Cleome* is evident, for in a marked degree the extreme variations in sex are independent of any other visible change and the various grades of intersexes are present from the beginning of bloom.

Furthermore, the influences that affect the sex of a single flower often extend to groups of flowers. Thus there is a period of maleness, which is followed by a period of femaleness or of bisexualism, and this in turn is followed by maleness. Flowers in the same condition as to sex are grouped along the raceme. There is a series of cyclic changes all occurring during the period of continuous bloom.

These qualitative changes in sex in flowers of *Cleome spinosa* do not involve the transformation of organs of one sex into organs of the other sex after differentiation has begun, as is the case in many of the intersexes reported in animals (Goldschmidt and Poppelbaum, 1914; Goldschmidt, 1916; Banta, 1916; Lillie, 1917; Sexton and Huxley, 1921). Here the change is accomplished, as it is in dioecious plants, by the abortion of one or the other kind of sex organs. The relative position of each in the flower as a whole is maintained, but the differentiation giving male and female flowers (along with bisexual flowers) is as complete as is seen in many species of dioecious plants. The differential determination of sex in repeated cyclic alternative changes as they occur in *Cleome spinosa* shows to what degree the internal correlative differentiations in development may be extended to the organs of sex after the plant as a whole has passed from the exclusively vegetative to the reproductive stage. At the time of the transition to the reproductive stage, the change is not necessarily complete and discontinuous, nor are the flowers produced in succession necessarily of the same grades of sex. Even when the flowers appear to be morphologically the same there may be a decided cyclic change in their physiological character, as is the case with *Brassica chinensis* and *B. pekinensis* (Stout, 1922). The contrast between these species of *Brassica* and *Cleome spinosa* illustrates well the different types of sterility that may develop in plants and the different expressions of cyclic regulation of them. In these *Brassic*as there is frequently rather decided abortion of flowers at the time of transition from vegetative to reproductive organs; in *Cleome* no indication of such abortion is present, the first flowers to appear being often fully developed as hermaphrodites. In the *Brassic*as there is a somewhat extended period of flower formation with flowers all morphologically bisexual—but in which the physiological relations in fertilization may vary in a very definite and single cycle; in *Cleome spinosa* there is no variation in the physiological nature of stamens and pistils that are at all functional in so far as these may be tested by the relations of fertilization, but there is the cyclic alternation in the morphological development of the organs of sex. This comparison illustrates two rather widely different expressions of sex in its relation to fertility and sterility.

The conditions in *Cleome spinosa* favor the view that, as held by Yampolsky (1920), there is a general tendency away from hermaphroditism toward dioecism among the higher plants. In the persistence of perfect flowers in greater or less numbers along with those which are more or less

purely staminate or pistillate, *Cleome spinosa* is like most species which are in the transition stages toward dioecism. The alternate appearance of male, female, and hermaphroditic flowers in a raceme of course favors crossing, and when this alternation tends to be synchronous on all the branches of a plant, selfing is largely prevented except in the case of the hermaphroditic flowers.

In the spider flower, with its long flowering period and its alternation of maleness and femaleness in the racemes, it is evident that practically the whole vegetative feeding power of every plant is drawn upon for seed production. The conditions are markedly different, and we may consider them more highly adaptive to the demands of reproduction, than is the case in strictly dioecious plants in which seed production is confined to one of each pair of male and female plants. We may, perhaps, characterize the sex conditions in *Cleome spinosa* as effecting a sort of *super-dioecism* in that the conditions favor both reproduction and crossing for each individual.

Certain points regarding the determination of sex in the flowers of *Cleome spinosa* are clear. The conditions illustrate well the fact that the morphological differentiations of sex are fundamentally an extension of the phenomena of somatic differentiations. The expressions of differential qualities in leaves, stems, and flowers, with further differentiation of calyx, corolla, pistil, and stamens, with still further differentiations of tissue within each, are all recognized as one-sided, qualitative, and alternative expressions in protoplasmic units that are alike and which still remain alike in fundamental constitution. Even the physically qualitative division of germ plasm in the reduction divisions is found in regeneration experiments and in parthenogenesis not to be a direct and absolute condition in the alternation of generations. The theory of sex chromosomes decidedly fails in general application to plants, and even in animals, where its application seems most marked, sex is often intergrading and reversible, showing that there is alternative expression rather than alternative inheritance.

In *Cleome spinosa* it is evident that there are rather special and perhaps very specific stimulating and inhibiting influences which regulate the development of the sex organs. Whether these influences are substantive or more of the nature of stimuli, their action is cyclic and decidedly alternative. The results clearly show that sex of flowers is determined progressively as they are formed in response to regulation by internal biogenetic conditions.

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EXPLANATION OF PLATE VI

Each group of flowers is from the same raceme on the same date. Petals have been removed. The scale in inches shows the reduction from natural size.

FIG. 2. Three sister flowers uniform as to maleness but highly variable in femaleness.

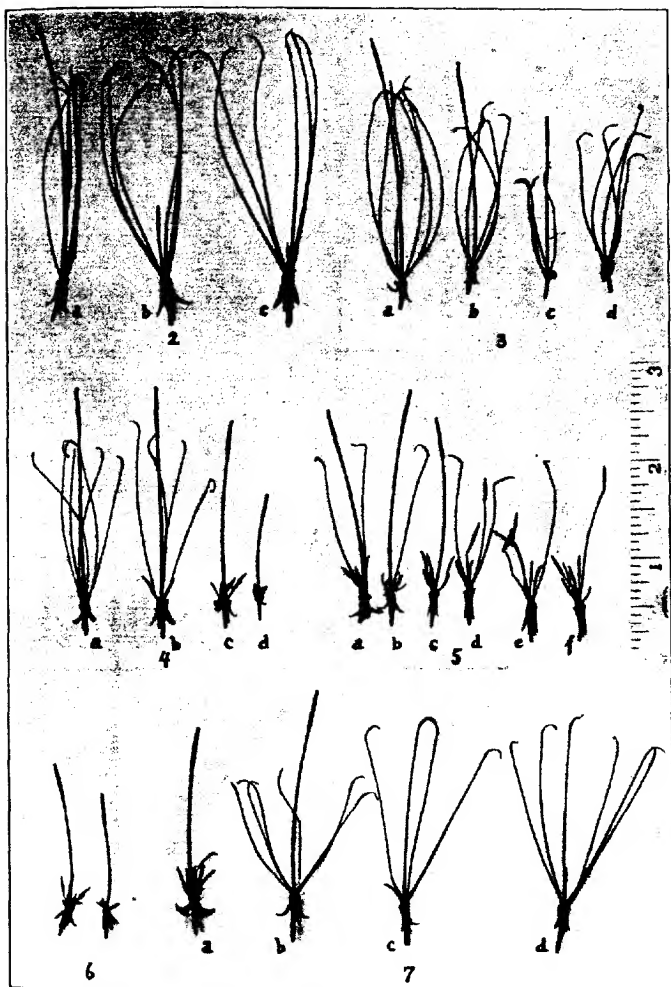
FIG. 3. The stamens of the four flowers vary as to length; one stamen in *b* and one in *c* are aborted; all others are highly functional. Pistils are functional in *a*, *b*, and *c*, but aborted in *d*.

FIG. 4. Four flowers from same plant as those of figure 3, several days later. Pistils vary as to length but all are functional. Stamens all excellent in *a*, two much aborted and indehiscent in *b*, all indehiscent in *c*, and all much aborted and containing no pollen in *d*.

FIG. 5. Pistils variable; in *a*, *b*, and *c*, fully functional; in *d*, *e*, and *f*, rather aborted. Stamens highly variable in each flower irrespective of the condition of the pistil.

FIG. 6. All stamens much aborted or rudimentary. Pistils somewhat undersized but functional.

FIG. 7. Abortion of stamens only, as at *a*, or of pistil, as at *c* and *d*, with a flower fully hermaphroditic; all in bloom at the same time on the same raceme. Illustrates well the marked one-sided abortion of sex organs.



SIOUT: ALTERNATION OF SEXES

INTERNAL DECLINE OF LEMONS

I. DISTRIBUTION AND CHARACTERISTICS¹

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(Received for publication April 12, 1922)

INTRODUCTION

The term "internal decline" has been applied to a physiological abnormality causing the destruction of internal tissues in the lemon fruit, usually in the stylar end. The term as here used includes "blossom-end decay," "tip deterioration," "yellow tip," "dry tip," and other terms all applied locally by growers, packers, and shippers to the same trouble. When the study of this malady was first begun, the term was applied also to a browning of the "core" (placenta) and of the membranes (inner carpellary walls) covering the pulp segments of the lemon. Now it is applied only to the abnormality having the characteristics described in this paper.

Internal decline has been known by the lemon growers of California for at least 25 or 30 years, and the indications are that it is increasing in severity. Its seriousness fluctuates from year to year. For example, in 1920 in many of the groves as much as 10 to 60 percent of the fruit in an entire pick had to be culled out and discarded or sent to the by-products laboratory, while in the same groves in 1921 not more than 2 to 5 percent had to be discarded.

Experimental studies on internal decline were begun by the junior authors in 1915, and have been carried on intensively by the senior author since the summer of 1920. His discussion concerning one phase of his work on this problem will appear in an early number of this journal as the second paper in this series.

GENERAL DISCUSSION

Distribution. Internal decline may appear in the fruit of almost any lemon grove in southern California, except that it seldom, if ever, occurs in groves located within a few miles of the coast. Groves situated within the hot inland valleys are especially likely to show a large amount of the trouble. A great variation in distribution may occur not only in different groves but in different parts of a given grove.

Varieties Affected. The two principal varieties of lemons grown in California are the Eureka and the Lisbon. Besides these there are a few other varieties which are grown in comparatively small numbers. Any of

¹ Paper no. 94, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

these varieties may be affected with internal decline. Data thus far obtained do not indicate any marked difference in varietal susceptibility. In some localities the Eureka is reported to be more susceptible while in other localities the Lisbon is said to suffer most, but so far the data do not confirm these reports.

Seasonal Appearance. The trouble usually appears with the beginning of warm weather in June and continues during the summer and fall until November or December. However, some lemons that are slow in attaining a desirable picking size may be allowed to remain on the trees for a longer period than usual, and consequently a few lemons showing internal decline may appear in the packing houses at almost any time during the winter or even in the early spring months.

Age of Trees. Internal decline has been found on trees of all ages ranging from 3 or 4 years up to 50 years. It is often the case that the fruit on young, thrifty trees with heavy foliage is worse affected than that on older trees with less dense foliage.

Age of Fruit. The terms "tree-ripe," "silver," "light-green," and "dark-green" are used in the lemon industry to designate lemons in different stages of maturity as indicated by their color. Tree-ripe lemons are those which remain on the tree until they have attained their mature yellow color; silver lemons are those which are picked at a time when most of the chlorophyll has disappeared from the fruit, leaving it a very light greenish yellow; light-green and dark-green lemons are picked according to standardized sizes. They are younger than the preceding kinds but are sufficiently mature for picking. When lemons are picked while they are yet green, they are either allowed to color naturally while in storage or are artificially bleached before being placed on the market. The lemons that are picked while green possess the most desirable commercial qualities because they have a higher acid content and are less susceptible to the attacks of diseases.

In some cases internal decline is found only in the tree-ripe fruit, but it often appears also in the silver, light-green, and dark-green fruit from the same grove. The tree-ripe fruit usually shows the greatest percentage of the trouble, sometimes as much as 95 percent being affected. However, in "bad years" as much as 60 percent or more of the green fruit in a given pick may be affected.

SYMPTOMS

Green Fruit

External Symptoms. It is often impossible to determine without cutting the fruit whether or not it is affected. In some cases the trouble may be detected by a partial loss of luster at the styler end. Another indication is the development of a yellow or orange-yellow color on a quarter or more of the styler end while the remainder of the lemon is still green; even this appearance, however, is not a sure indication of internal decline.

Internal Symptoms. The first internal symptoms are usually found near the vascular bundles in the "nipple" of the peel at the styler end of the lemon. Small cylindrical openings are found arranged in a circle within the ring of vascular bundles. It is evident that these openings have been produced by the collapse of the parenchymatous cells. A little later in the development the vessels themselves appear to be clogged with a pinkish to rust-brown deposit of gum. At this stage the vessels and the surrounding tissues often begin to break down, and finally the central portion of the peel of the styler end of the lemon becomes a mass of gum having the characteristic pink to rust-brown color. In other cases the vessels are clogged with gum but there is very little indication of collapse in these or adjoining tissues. This is true for the examples shown in Plate VII. In conjunction with these conditions, pink to rust-brown splotches frequently appear at one or more places within the adjoining tissues (see c, Pl. VII). The tissues in these splotches, at this time, usually have a glossy appearance due to the formation of gum. At this stage the cells and the juice sacs of the pulp adjoining the peel at the styler end of the lemon become affected, lose water, and collapse (see b, Pl. VII). There is very little or no discoloration of these tissues at this time. In comparatively rare cases the vessels in the center of the lemon are discolored and filled with gum all the way through to the stem end. This is usually as far as the trouble has progressed in lemons that are picked while light or dark green in color.

Silver Fruit

External Symptoms. Internal decline is not easily detected by external signs at this stage of development of the fruit. In many cases the more intensive coloring of the peel at the styler end of the lemon is an indication that the abnormal breaking down of the internal tissues has begun. However, as is true with the green fruits, this indication often may be misleading.

Internal Symptoms. The loss of water and the collapse of the pulp cells and juice sacs at the styler end continue. The progress is more rapid near the center of the lemon than out near the peel. It is especially rapid in the pithy core (placenta) that runs through the center of the lemon (see Pl. VII). As the fleshy pulp tissues dry out they may retain their normal color, but more often they assume a pinkish or light-brown color. In this stage of growth of the lemon it is found that, in those having internal decline, the breaking down of the tissue has progressed so far as to involve parts of the inner portion of the peel and perhaps a fifth of the adjoining pulp at the styler end.

Yellow (Tree-ripe) Fruit

External Symptoms. Here again the signs are far from being infallible. The more intensive yellow or orange-yellow coloring of the styler end, which may occur while the remainder of the fruit is still green, persists

after the fruit has become ripe and serves in many instances as an indication of internal abnormality. If the peel of the lemon is comparatively thin, the breaking down of some of the internal tissues will cause the formation of a depression at the base of the nipple on the stylar end of the lemon. In some cases the depression appears on one side only, thus causing the nipple to curve or bend over in that direction. These depressions are often, but not always, a sign that the lemon is affected and that it should be discarded.

In all the three classes of fruits the external signs are such that usually 50 to 90 percent of the lemons having internal decline may be detected and culled out.

Internal Symptoms. There is little or no indication of a further breaking down of the cells in the peel, but the pulp tissues continue to lose water and collapse as long as the fruits remain on the trees. When $\frac{1}{3}$ to $\frac{1}{2}$ of the stylar end of the lemon has become affected (see *d*, Pl. VII), an abscission layer usually forms in the stem and the fruit drops.

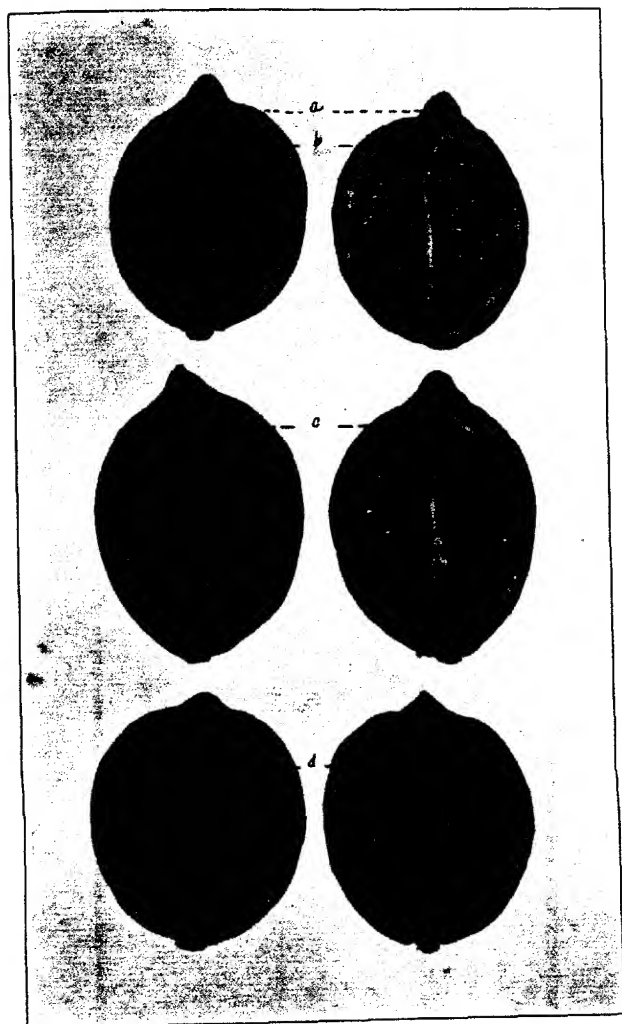
After the trouble has progressed to a considerable extent, the stylar end of the lemon becomes lighter in weight than the opposite end. On this account many of the badly affected lemons may be distinguished and culled out at the time of washing, because these lemons, unlike the sound ones, will float with the affected ends upward.

In the above description of the symptoms of internal decline, a typical example has been cited in which the collection of gum and the destruction of the tissues begin while the fruit is still green. As has already been stated, the malady may appear only in the silver or tree-ripe fruit. In such cases the course of development of the abnormal conditions is like that which begins while the fruit is still green, except that in some instances gum formation may be a little less abundant and discoloration of the tissues less pronounced.

EXPLANATION OF PLATE VII

- a. Vascular bundles clogged with gum.
- b. Initial collapse of pulp tissues.
- c. Affected area in peel: vessels and adjoining cells becoming filled with gum.
- d. Collapse of placental and adjoining tissues. The lemon usually falls from the tree at about this stage.

Note: The earlier stages of development of the malady cannot be satisfactorily shown in an ordinary photograph, and in fact none of the stages can be most advantageously shown without the use of a colored plate.



BARTHOLOMEW AND OTHERS: INTERNAL DECLINE

THE RELATION OF TEMPERATURE TO THE FUSARIUM WILT OF THE TOMATO¹

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(Received for publication April 19, 1922)

There are three different tomato diseases in the United States, supposed to be caused by three different species of *Fusarium*. They are the "summer blight" of California, the "yellow blight" of the Pacific Northwest, and "Fusarium wilt," which is one of the most serious tomato diseases in the southern states. Each of these geographical sections is subject to exceedingly high temperatures, and it has been observed that in periods of very high air and soil temperatures the *Fusarium* diseases cause the most serious damage. Edgerton (3) has recently published his observations on the relation of high temperatures to the *Fusarium* wilt disease. It is for this disease that the writer has attempted to determine definite temperature limits. The work herein described was carried on entirely under greenhouse conditions in the "Wisconsin soil-temperature tanks," the primary object being to study the effects of (1) different soil temperatures and (2) different air temperatures upon the development of the disease. The influence of other environmental conditions on the development of the disease, and detailed observations, hitherto unrecorded, as to the nature of the disease have been described also.

THE FUNGUS

The causal organism of the wilt disease under discussion is a vascular parasite of the tomato (*Lycopersicum esculentum* Mill.) which may live and retain its pathogenicity in the soil for considerable periods. Pure cultures of this fungus, designated as *Fusarium lycopersici* Sacc., were obtained from S. H. Essary of Tennessee, and in addition, isolations were made by the author from infected soil and infected plants sent from Maryland, Tennessee, Ohio, and Indiana.

When inoculations were made with these various isolations, it was found that, under greenhouse conditions at least, some produced much more serious wilting than others. After a series of preliminary inoculation experiments, therefore, one Indiana isolation was chosen for the temperature experiments. This strain was not so virulent a parasite as a Maryland isolation, but it produced the disease under a wide range of temperatures and was about equal in virulence to several other strains from Indiana,

¹ Investigations carried on at the University of Wisconsin. The work was done under advisory relations with Professors L. R. Jones and E. J. Kraus, to both of whom the author expresses his indebtedness for suggestions and criticisms.

Ohio, and Tennessee. In order to make sure that this Indiana strain chosen was representative among these isolations when inoculated plants were grown at different temperatures, a number of tomato plants were inoculated with it, an equal number with the Tennessee strain, and several pots of each were grown at several temperatures. The results in the two series were remarkably uniform. The amount of disease produced at each temperature was nearly equal in plants inoculated with the Indiana strain and in those inoculated with the Tennessee strain. Also, when pure cultures of the two strains were incubated together at different temperatures (for methods, see page 78), their respective optimum, maximum, and minimum temperatures for growth were found to be the same.

Identification. In order to insure confidence as to the identity of the organisms in hand, morphological and cultural studies were made following the methods of Sherbakoff (12). The Indiana strain was made the basis of these, with some comparative studies upon the other strains. The spore counts and measurements were made from 15-day-old cultures grown at 28° C. The following media were used: clover stems, tomato stems, potato plugs, rice, beans, potato agar (both 2 and 5 percent glucose), oat agar, malt agar, and water agar. It was found, however, that all the vegetative and reproductive parts of the fungus and all the color changes produced in any of these media were found on rice, tomato stems, and oat agar. The identification work consisted of observations as to the color and type of growth, the production and appearance of sclerotia and sporodochia, the character, septation, and size of macroconidia, and the method of bearing the chlamydospores.

These studies justified the conclusion that the organism in hand was *Fusarium lycopersici* Sacc., and that, although there are minor variations in pathogenicity and other characters, the *Fusarium* wilt disease of the eastern United States is probably all attributable to this one species.

TEMPERATURE RELATIONS OF THE FUNGUS IN PURE CULTURE

In order to determine the range of temperatures through which *F. lycopersici* grows vigorously, pure cultures of the Indiana strain were prepared as follows: A small drop of a spore suspension made from a single-spore culture was placed in the center of each of a series of Petri dishes containing potato hard agar. These Petri-dish cultures were incubated at 28° C. for 24 hours and examined under the low power of the microscope. All cultures in which no spores had germinated, or which contained more than two or three germinating spores, were discarded, and three of the remaining dishes were incubated at each of 12 graduated temperatures, ranging from 4° to 38° C. It was found by daily observation that after six to eight days the fungous growth at temperatures approaching 28° C. entirely covered the surface of the dish. Plate VIII shows representative Petri-dish cultures after incubation for five days at each of the temperatures

chosen. As indicated by this photograph, the minimum for growth is 9–10° C., the optimum about 28° C., and the maximum 37° C., when the amount of growth is measured by the diameter of the colony. This trial was repeated with like results.

Similar cultures were made to determine the relation of temperature to size and septation of spores, the detailed results of which are not pertinent to this discussion. It was found, however, that, even when a single-spore strain of the fungus was used, there were marked differences in the average size of the spores as well as in the number of septa in the spores obtained from cultures grown at different temperatures.

TEMPERATURE RESPONSES OF THE NORMAL HOST

Two commercial varieties of tomato, Mangus and Chalk's Early Jewel, both susceptible to the wilt disease, were used in these studies. In each experiment uninoculated plants were grown as controls under the same conditions as the inoculated plants. These controls afforded an opportunity to study the growth of the host at different soil temperatures. It was found that the temperature range which, in sterilized soil under greenhouse conditions, proved most favorable for a vigorous development of these varieties was from 24° to 31° C. This range includes the temperature for the optimum development of the fungus (28° C.). The growth of the uninoculated plants is illustrated in Plate IX, C, a photograph taken one month after the plants had been transplanted at soil temperatures of 19°, 22°, 24°, 28°, 31°, 33°, and 35° C. respectively. As stated above, the plants grew most vigorously at temperatures of 24° to 31° C. and somewhat less vigorously at 33° C., while at 35° they lived but did not increase appreciably in size. In the cooler soil, growth decreased gradually from 24° to 19° C. and below.

SYMPTOMS OF THE DISEASE

General Symptoms. The Fusarium wilt of the tomato as produced in the greenhouse at Madison was characterized by a progressive wilting of the leaves, often accompanied or preceded by a yellowing of the affected leaves. The wilting was sudden and permanent and developed most rapidly during bright, sunny weather, with little or no recovery during the night. The browning of the infected bundles could readily be seen in the cut stems. The roots appeared normal externally, though the bundles were browned as in the stems.

In well-developed plants three to four weeks old, the first external evidence of the disease was always the wilting of a lower leaf, this leaf being the first one supplied by the infected bundle or bundles. Frequently, one side of such a leaf wilted and the other side remained healthy for a time. In such instances, a cross section of the leaf petiole showed discoloration of the bundles on the wilted side only. The disease appeared next in other

leaves supplied by infected bundles, those leaves progressively higher up on the stem being attacked later. When, however, the fungus reached the succulent tissues of the upper portion of the plant, there was a sudden wilting of the entire plant. The stem and the older petioles were always the last to succumb and remained green and turgid for a time after the other parts had shriveled and blackened.

The symptoms varied somewhat, however, under conditions which were not favorable for the maximum development of the disease. Thus, if the plants were resistant, if the fungus was lacking in virulence, or if the environmental conditions were unfavorable to the growth of either host or parasite, the tendency was for the disease to appear as a slow blight rather than as a wilt. In this blight the leaves yellow and die slowly, while in the wilt they droop suddenly and die without yellowing.

Symptoms in Relation to Temperature. If a susceptible variety of tomato, such as Chalk's Jewel, is inoculated with a virulent strain of *F. lycopersici*, and the general growth conditions are optimum for the development of the disease, the following classification may be made on the basis of soil temperature:

First: Temperature optimum for the disease, *i.e.*, 25° to 31° C.—a sudden wilting which appears first in the lower leaves, then in those progressively higher up, and is rarely accompanied by yellowing of the leaves affected.

Second: Temperature just above or just below the optimum, *i.e.*, 33° to 34° C., or 20° to 24° C.—a wilting accompanied, and often preceded, by a yellowing of the leaves. The appearance may frequently be that of a slow blight rather than of a wilt for, as above stated, there is more yellowing than actual wilting, and the plants often show stunting of growth.

Third: At temperatures above 34° or below 20° C. there is no external evidence of the disease.

Fourth: In addition to the manifestations of disease mentioned above, the fungus may enter the host but penetrate the bundles in only the lower portions of the stem. This condition is often the result of a short exposure to temperatures favoring the disease, followed by a drop in temperature sufficient to check further development of disease. Plants thus infected are lighter in weight than uninfected plants grown under similar conditions, and the greater the amount of infection, the greater the loss in weight.

GROWTH DIFFERENCES OF HOST AND PARASITE IN RELATION TO THE DISEASE

Since a parasitic disease is the result of interactions between host plant and parasite, external influences which modify the appearance of the disease are necessarily effective through the changes which they produce either in the host, in the parasite, or in both. If those complexes, called host resistance on the one hand and virulence of the parasite on the other,

were equally increased or decreased by a change in temperature, the degree of disease-expression might remain the same even though fundamental changes had taken place. It is more reasonable to expect that changes in temperature would not equally increase or decrease both the attacking power of the fungus and the resistance of the host, or, in other words, that pathogenicity might be increased out of proportion to resistance, or *vice versa*. It is also quite conceivable that a temperature change (*e.g.*, from medium to low) might increase the resistance of the host and at the same time reduce the pathogenicity of the parasite.

Since the amount of disease produced in the case of the *Fusarium* disease of tomatoes did vary at different temperatures, it seemed possible that a study of the temperature relations of the host plant and of the fungus separately might aid in interpreting the variations in the amount of disease produced under the different conditions.

In so far as the host plant is concerned, differences in temperature would affect the processes of food manufacture and also the utilization of the elaborated substances in respiration and other activities. Some micro-chemical work was undertaken in an attempt to discover the gross differences in composition which might be correlated with differences in the amount of disease. Reducing sugars, starch, and nitrates were measured quantitatively. It was not presumed that these materials were necessarily directly associated with qualities of resistance, but they are intimately concerned in the processes of growth, and the quantities present at any particular time vary widely with conditions of nutrition and environment. They might serve also as indicators of still other materials, possibly derived from them, which are more closely associated with resistance.

No definite interpretation can be made of the results of these chemical tests. When the plants were grown at approximately 17° C. there was no disease, while at 27° C. there was a maximum of disease, yet the quantitative differences in the compounds tested for in the two sets of plants were not marked. Again, with conditions of warm soil and cool air the lower portions of the plants held at the warm temperature were readily invaded by the fungus, which did not penetrate the upper parts surrounded by cool air. The chemical analyses, however, did not indicate marked differences in the composition of the tissues of these two regions.

There is a closer correlation, perhaps, between the temperature ranges of the host, the parasite, and the disease, considered separately, in the case of the *Fusarium* wilt of tomatoes than is ordinarily found. This correlation is especially marked at the optimum points, *e.g.*, it has been noted that the temperatures at which the host makes its most rapid growth range from 24° to 31° C. The fungus also grows most rapidly at these temperatures, having its optimum at approximately 28° C., and the disease is most destructive at soil and air temperatures of 25° to 31° C. There are, however, differences. Thus, the disease develops more rapidly at 31° than at 25°.

while the fungus grows with equal rapidity at 24° and 31°. Again, the growth of the fungus at 33° to 34°, the upper limit for the disease, was much less vigorous than the growth of the fungus at 19° and 21°, the lowest temperature at which the disease occurs.

Both host and parasite, taken individually, develop at wider ranges of temperature than does the disease occasioned by their interaction, the minimum and maximum temperatures being 9° and 37° C. for the parasite and approximately 10° and 36° C. for the host, while for the disease the minimum is 20° and the maximum 33° C. The relative rate of development of host, parasite, and disease corresponds in a general way as the temperatures are raised or lowered.

THE CAUSE OF WILTING AND DEATH OF THE HOST

The cause of wilting and the subsequent death of *Fusarium*-infected plants is still undetermined. It has often been assumed that the fungus actually fills the xylem vessels of the stem and thus limits the passage of water through the plant. Recently, both Haskell (6), studying the *Fusarium* wilt of potatoes, and Brandes (1), in his observations of the *Fusarium* wilt of bananas, have concluded that death is due to toxic substances produced by the fungus. There is some evidence that a toxic substance may be produced in *Fusarium*-infected tomato plants.

If it is true that the wilting results from plugging of the xylem tubes, then it should be possible to reproduce the symptoms by cutting away tissue, including xylem elements, in the corresponding parts of a healthy plant. As a matter of fact, although it is true that when one side of the leaf is diseased the infected vascular bundles are found only on the diseased side, yet, if the petiole of a healthy leaf is cut half-way through, the injured side does not wilt. There seems to be sufficient lateral diffusion of water to keep the entire leaf turgid. Similarly, leaves on one side of a stem can not be made to wilt by cutting through the vascular bundles on that side of the stem.

In Plate IX, A, is shown a plant which has thrown out vigorous suckers from an old diseased stem. This plant was transferred from a temperature of about 27° to one between 15° and 20° C. when the disease was in an advanced stage. The fungus was recovered from the very tip of the plant at the time of the transfer. All the leaves and the growing tip were killed, but in the cool temperature the old roots and stem were able to supply the moisture and other nutrient materials for a new top, which grew rapidly and appeared healthy. If the old stem had wilted because of plugging of the xylem tubes, it is difficult to believe that the change in temperature could have relieved this condition so rapidly. But while the temperature change would not affect the structural relations within the old stem, it would immediately influence such physiological reactions as the formation and activity of an injurious substance and the ability of the host tissue to neutralize it.

The type of wilt characteristic of the *Fusarium* disease also seems to differ from purely mechanical wilting in that there may be a complete and rapid killing of tissues and in that cells may regenerate and produce new shoots. In these respects it is more nearly comparable to the injuries produced in plants by the injection of poisonous compounds.

Free (4) has noted that the effects of poisonous compounds applied to the soil are much localized in the plant, and he suggests that this is due to the accumulation of these compounds in certain parts.

Of more direct interest are the experiments of Rumbold (10), in which toxic substances were injected into the trunks of chestnut trees. She found that

The affected region extended up and down the trunk in a line whose width usually was but little more than the injection hole. The cells through which the solution passed acted like a blotter, with the result that the farther from the point of injection the more dilute was the solution and the smaller the injection stream.

And again, in a later publication (11),

When a "killing" solution was injected the path was marked on the bark by vertical strips of dead tissues. Those twigs and branches whose vascular system entered this path were killed; often but one side of the branch was affected.

Gray (5) has found that many substances produce toxic effects when injected into tobacco stems. He found that in this herbaceous plant the localization of effects was quite as striking as in the case of the chestnut. Thus, a poisonous substance injected into one side of the stem passed up the stem rapidly and affected the leaves directly in its path; the lateral diffusion, however, was very slight. It was very common to find half a leaf killed and the other half remaining healthy, this effect being readily produced by injecting the poison just below and at one side of the petiole. In all cases there was marked discoloration of the bundles; and only parenchymatous tissue in immediate proximity to these browned bundles showed the toxic effect.

Thus the toxic effects of injection of poisonous substances and the killing of host tissues caused by the invasion of *F. lycopersici* appear very similar. It has been demonstrated by experimental work that with a warm soil temperature and a cool air temperature the infection of the plants takes place readily, the entire vascular system of the tap root and lower stem becoming infested with fungal hyphae and badly discolored. The cool air, however, prevented the fungus from extending up into the aerial parts, which remained apparently healthy both externally and internally, the plants making an excellent growth.

As to the nature of this toxic substance, there are several possibilities. It may be that it is a specific secretion of the fungus, or it may be a substance formed through the interaction of the host and the parasite. It has been amply demonstrated that even so-called normal root excretions removed in leaching may stunt plants grown in soil watered with these leachings.

With this disease, since the fungus is growing in the xylem vessels, toxic products could be promptly carried to the leaves.

To consider the manner in which the toxin affects the host, as a starting-point there is the fact that a wilt is produced. Caldwell (2), discussing wilting, says:

Permanent wilting may result from the operation of either one or both of two factors: (a) decrease in the soil moisture content, (b) loss of water from the plant by transpiration. Attainment of the permanently wilted condition indicates a certain degree of reduction in the amount of water contained within the organism.

The primary effect of a toxic substance such as has been supposed to exist here would probably be upon the permeability of cell membranes. The permeability of the membranes might be increased, in this case facilitating loss of water from the cells, and, as a result, there might be a rapid increase in the rate of transpiration from the leaf as a whole. On the other hand, it is quite possible that permeability is decreased and that there is a slowing up of the movement of water from cell to cell. As a result of this, those cells farthest away from the veins might dry up completely while cells adjacent to the veins remained turgid.

Livingston (8) has shown that permanent wilting may occur with the stem and roots functioning normally, when the rate of transpiration from the leaves is greatly increased.

METHODS OF EXPERIMENTATION

The experiments here described were all conducted in the greenhouses at the University of Wisconsin, and the Wisconsin temperature tanks were used in all cases in which soil temperature was controlled. In these tanks cylindrical cans of galvanized iron are immersed in water, the temperature of which is controlled by an electrical heating device. The most uniform infection occurred when a good loam soil was sterilized by steaming for three hours at six pounds' pressure and subsequently inoculated with *Fusarium lycopersici*. Neither the same loam not sterilized but artificially inoculated, nor imported naturally infected soils gave such consistent infection as was secured when soils were first sterilized and then inoculated. Unless otherwise indicated, freshly sterilized soil was inoculated by mixing with it a quantity of the mycelium of the fungus. For this purpose the fungus was so grown on rice in 500-cc. Erlenmeyer flasks as to obtain an abundant mycelial growth. The inoculated soil was then incubated at about 25° C. for a week or ten days in order to give the fungus time to permeate thoroughly and uniformly the soil mass. The galvanized-iron containers used in the Wisconsin temperature tanks were partially filled with sterilized soil, the upper four inches were filled with the inoculated soil prepared as described above, and the whole was thoroughly mixed. These containers were then incubated for several days at the temperatures to be maintained throughout the experiment. Thrifty tomato plants (4-5 inches

high) of the Mangus variety or Chalk's Early Jewel were transplanted into the containers, one plant of each variety being set in each container. Usually six such pots were held at each temperature until the infected plants at the optimum temperature for the disease were completely wilted. This time was a month or more, and meanwhile the progress of the disease was noted daily.

The plants used in these experiments were grown in three-inch pots until they were transferred to the inoculated soil. In transplanting, no considerable amount of soil was removed from the roots by washing or other methods, but the whole root system was kept as nearly intact as possible. A few of the smaller roots were necessarily injured, but infection was not more abundant in the plants bearing such roots than in those inoculated in the pots in which they were grown, and which, therefore, had no rootlets injured by transplanting.

SOIL-TEMPERATURE EXPERIMENTS

1. *Experiments in which the air temperatures were uniform and the soil temperatures varied*

Three experiments, which for convenience may be referred to as I, II, and III, were conducted at a uniform air temperature and different soil temperatures. Only one of these experiments, however, need be described in detail, as the responses of the disease to different temperatures were the same in all trials.

Experiment III. Nine different tanks held at temperatures of 19°, 21°, 22°, 23°, 24°, 28°, 31°, 33°, and 35° C., respectively, were used in this experiment. Each tank accommodated six culture cans with two tomato plants in each can; three of these cans contained inoculated, and the other three uninoculated soil.

TABLE 1. *Results showing percentages of infection which occurred in plants grown in uniformly infected soil and held at soil temperatures ranging from 19° to 35° C. (Experiment III); when leaves were wilted and bundles blackened in stems, roots, and leaves, plants were counted as infected.*

Treatment				Results	
Temperature ° C.	No. Plants Used	No. Plants Infected	No. Days before 1st Infection	No. Days before Last Infection	Average No. Days before Infection
19.....	6	1	29	—	29.0
21.....	6	0	—	—	—
22.....	6	5	22	29	25.0
23.....	6	4	21	29	25.5
24.....	6	5	20	28	23.0
28.....	6	6	12	22	14.5
31.....	6	6	10	22	15.5
33.....	6	1	26	—	26.0
35.....	6	0	—	—	—

From this table it appears that the optimum soil temperature for the development of the disease is 28° to 31° C. (82° to 88° F.), the minimum 22° C. (71° F.), and the maximum 33° C. (91° F.). In this series one infection occurred at 19° C. but none was found at 21° C., a temperature at which one isolated infection had been secured in a previous experiment. The temperatures from 22° to 32° C., inclusive, resulted in the infection of about the same number of plants, the most favorable temperature for infection being distinctly marked, however, by the quickness and virulence of the attack. At 33° C. only one plant was infected, and at 35° C. none were infected.

The growth of the check plants is illustrated in Plate IX, C. The tops there shown are representative of the condition of the check plants one month after they had been transplanted to the temperature pots. At 35° C. these check plants maintained themselves and increased in size very slowly; 33° C. was a slightly more favorable temperature, and at 31° C. the plants grew luxuriantly. The growth at 28° and 24° C. was almost as great, and these plants appeared to be growing most rapidly at the time the experiment was stopped. The plants were growing thriftily at 22° , and equally well at 21° , while at 19° a slight decline was apparent.

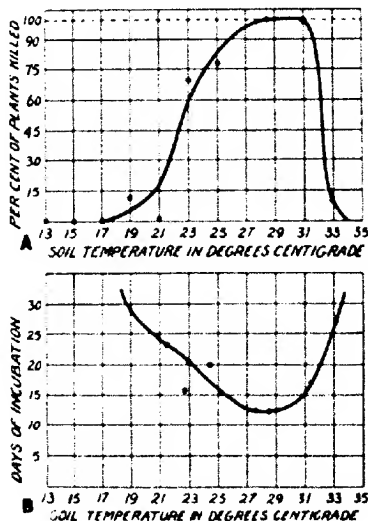


FIG. 1. The data for graphs A and B were secured in soil-temperature experiments II and III. Graph A shows the relation of soil temperature to the percentage of plants killed. The plants were exposed to the different temperature conditions for one month. Graph B shows the relation of soil temperature to the incubation period in days. Thus, it took much longer for the disease to appear with the soil at a temperature of 22° to 23° C. than with it at a temperature of 27° to 30° C.

These experiments showed, then, that there are ranges of soil temperature, approximately 14° to 20° C. and 34° to 35° C., which permit growth of both the plant and the fungus separately, but which do not favor the parasitic development of the fungus within the host.

2. *Experiments in which both air and soil temperatures were varied*

Early in these studies it became apparent that, although this fungus entered the plant through the root, it made its way into the aerial stem and did its damage in the above-ground parts. The roots appear to die last, the sequence being: leaves, then the main stem, and finally the roots. From previous work with soil temperature, the conclusion was drawn that soil temperature is a controlling factor only so long as the fungus is in the soil or roots, and probably exerts little or no influence after the fungus has grown up out of the soil. Thus it seemed logical to suppose that, once the plant parts above ground are diseased, air temperature alone might influence the progress of the wilt. In order to determine whether or not this is the case, experiments were conducted in which air temperatures as well as soil temperatures were varied.

Methods. Three sections of greenhouse were used in these experiments, with air temperature controlled at approximately 17°, 28°, and 33° C., respectively, by methods to be described in detail in a later publication. Unfortunately the ranges of air temperature procured were subject to considerable fluctuations, since in chambers where the bright sunlight is not counteracted in some way no ordinary means will prevent a rise in temperature. In reality, three ranges of temperature were obtained: a cool range of from 15° to 25° C., a warm range of from 25° to 30° C., and a hot range of from 30° to 35° C. In each of these sections a set of three constant-temperature tanks was placed. These tanks were maintained at 17°, 27°, and 35° C., to correspond with the air temperatures. Thus, by controlling both air and soil temperatures, nine combinations of soil and air temperatures were obtained (table 2).

Two experiments were conducted under these conditions of temperature. In Experiment IV the soil was steam-sterilized as usual and placed in the temperature-tank containers, and a spore suspension of the fungus was poured on the surface. Mineral wool, packed closely around the stems of the plants after they were set, served as insulating material. Two well-developed plants were used in each can.

In Experiment V, very small seedlings were transplanted into the sterilized soil of the containers and allowed to grow at 15° to 18° C. for a month, after which period the containers were transferred to the temperatures to be used throughout the experiment and incubated there for several days before the inoculum, a spore suspension, was added. A layer of mineral wool over the surface of the soil was used for insulation. At the beginning of the experiment the seedlings had attained a height of 3 inches and were

in a vigorous, thrifty condition. With two containers in each tank and three plants in each container, there should have been 6 plants at each temperature. However, the plants in one of the tanks died (table 2).

In each experiment plants were grown for one month in inoculated soil at the temperatures above stated.

Results of Experiment IV. Of the cool-soil-temperature plants, those grown in cool air and hot air (17° and 35° C.) showed no vascular discoloration; the isolation results also were negative. The plants grown in cool air (17° C.) and warm soil (27° C.) showed very badly discolored bundles at the base of the stem. This discoloration extended only a short distance up the stem, however, the entire stem which was surrounded by the cool air appearing perfectly normal externally. When the discolored bundles were plated out and incubated for a few days, an abundant growth of *Fusarium lycopersici* was secured in every case.

Plants grown in warm air (27° C.) and cool soil (17° C.) showed no evidence of vascular discoloration when sectioned. However, when the tissue from the base of the stem was plated out from each of the four plants, the fungus was recovered in pure culture from one of them. Warm air (27° C.) and warm soil (27° C.) gave 100 percent of disease, though only half the plants were completely dead at the conclusion of the experiment. Warm air (27° C.) and hot soil (35° C.) gave no evidence of vascular browning; the isolation results were also negative.

Hot air (33° C.) and cold soil (17° C.) produced neither disease nor vascular discoloration, but the fungus was obtained in pure culture from the base of one plant. Hot air (33° C.) and warm soil (27° C.) produced the disease in virulent form, one plant escaping infection while the other three were diseased or dead at the conclusion of the experiment. No results were secured with hot air (33° C.) and hot soil (35° C.), the plants dying soon after they were set in the pots.

As to the incubation period, the disease made its appearance several days earlier with the hot air (33° C.) and the warm soil (27° C.) than with the warm air (27° C.) and the warm soil (27° C.). The rapidity of the development of the disease, however, was about the same with these two combinations.

Results of Experiment V. The results of this experiment duplicated those of Experiment IV in the general temperature relations. However, the development of the disease was more marked than in Experiment IV because of the prevalence of bright, sunny weather.

At the end of the experiment all plants were closely examined for external evidence of disease. They were also sectioned to determine whether or not vascular infection had taken place. As table 2 indicates, under the heading "Discoloration of Vascular Bundles," infection of the xylem elements occurred in at least some of the plants at each temperature; but only when the infection progressed into the stem above ground were the usual

TABLE 2. *A résumé of the results of Experiment V, in which tomato plants were grown for one month in inoculated soil. In this experiment, as in Experiment IV, both air and soil temperatures were controlled.*

Temperature		Discoloration of Vascular Bundles	Wilting	Incubation Period of Disease	Comparison of Green Weight of Inoculated Plants with that of Check Plants
Air	Soil				
17° C.	17° C.	3 plants none 3 plants traces	None	—	—
17° C.	27° C.	6 plants marked	None	—	— 19%
17° C.	35° C.	3 plants none 3 plants traces	None	—	— 6%
27° C.	17° C.	3 plants none 3 plants traces	None	—	— 8%
27° C.	27° C.	2 plants marked	Marked	13 days	— 72% (4 plants out of 6 killed)
27° C.	35° C.	3 plants none 3 plants traces	Traces	20 days	— 28%
33° C.	17° C.	4 plants none 2 plants traces	None	—	+ 12%
33° C.	27° C.	3 plants marked	Marked	10 days	— 62% (3 plants out of 6 killed)
33° C.	33° C.	4 plants none 2 plants traces	Traces	25 days	— 28%

wilting symptoms observed. For example, when the soil temperatures were warm (27° C.) and the aerial parts were surrounded by cool air (17° C.), there was heavy discoloration at the base of the stem which neither progressed far above the soil line nor resulted in the usual wilting. The effect of the disease on the green weight, given in the last column of table 2, was calculated by using as 100 percent the green weight of tissue produced by a similar number of uninoculated plants grown under like conditions of temperature. As may be seen from the table, only two of the nine combinations of soil and air temperature so favored the development of the disease as to result in the death of plants. When soil temperatures were low (17° C.), infection was always slight; at the higher soil temperatures, when infection was abundant, it did not progress into the aerial parts of the plant if those parts were surrounded by cool air. On the other hand, when the soil temperatures were high enough (27° C.) to favor infection and the air was warm enough (27° or 33° C.) to favor the spread of the fungus through the aerial parts, the plants were badly diseased. Contrary to results obtained in previous experiments, the plants grown at soil temperatures of 35° C. and air temperatures of 27° C. showed some external

symptoms of disease. The symptoms, however, were not marked, consisting simply in a yellowing of the lower leaves, from which isolations of the fungus were made.

THE INFLUENCE OF ALTERNATION OF TEMPERATURE ON THE DEVELOPMENT OF THE DISEASE

All through these experiments it has been observed that, while the wilt developed with a fair degree of rapidity when the temperatures were controlled, it was during bright, sunny days when the temperature of the house rose considerably that the disease was most virulent. Since this was noted with plants grown under conditions of constant soil moisture, the wilt cannot in this case be associated with water shortage due to drying out of the soil. Likewise, from the experiments with high air temperature it can be definitely stated that continuous exposure of the plants to air temperatures as high as those attained during the bright weather is not effective in increasing the amount of wilt. In the high-temperature (33° C.) plants, just as in the warm-temperature (27° C.) plants, the disease developed slowly during cloudy weather and with great rapidity during bright days.

Table 3 illustrates the degree to which the virulence of the disease is accentuated by clear, bright weather.

TABLE 3. *The Relation of Sunlight to the Amount of Wilting of Tomato Plants Produced by Fusarium lycopersici*

Date	Weather Conditions	No. Plants which Wilting during Day
4 21	Clear	0
4 22	Cloudy	5
4 23	Cloudy	4
4 24	Clear	7
4 25	Partly cloudy	5
4 26	Cloudy	1
4 27	Cloudy	0
4 28	Clear	6
4 29	Partly cloudy	14
4 30	Partly cloudy	8
5 1	Clear	9
5 2	Clear	16
5 3	Clear	17
5 4	Cloudy	0
5 5	Clear	22
5 6	Clear	43

From this record, kept during one of the soil-reaction experiments, it will be noted that on clear or partly clear days the disease was much more active than on cloudy days.

The retardation of the development of the disease during dark weather

and the rapid acceleration during bright weather have occurred consistently throughout the work. Thus, while during the dark month of January plants which were inoculated and held at a temperature of 27° to 30° C. developed the wilt and died, the incubation period was several days longer, and the progress of the disease was not so rapid as with plants held at the same temperature range in April.

From these observations it would seem that with a constant temperature conditions most favorable for the development of the wilt can not be attained. While soil and air temperatures of 27° to 30° C. seem optimum for the wilt disease, it is actually true that with a constant temperature of 27° to 30° C. and a sudden rise in temperature of 4° to 5° associated with bright sunlight, the wilt develops the most rapidly. It is, of course, entirely possible that the strong sunlight might have a direct effect quite apart from the temperature influence. However, for the present the problem will be considered only from the standpoint of temperature.

Experiment VI. In order to observe further the influence of alternation of temperature on the development of the disease, at the conclusion of the air-soil temperature experiments a lot of twenty plants which had been growing in inoculated soil at a low temperature for two months was brought into the medium temperature house and allowed to remain there for ten days. The plants all appeared healthy at the end of this time. They were then divided into two lots, of which Lot 1 was allowed to remain always at the medium temperature (about 27° C.) while Lot 2 was carried into the high-temperature (33° C.) house during bright, sunny days, in order that it might be exposed to excessive air temperatures for short periods toward the close of the normal incubation period. The normal incubation period under these conditions would be about 16 days. The results were as follows:

The incubation period of Lot 2, occasionally exposed to high air temperature, was two days shorter than the incubation period of Lot 1, which was maintained at a constant medium temperature (27° C.). The plants of Lot 2 wilted quickly, the progress of the disease being much more rapid than that in Lot 1. Thus, one week from the date of the first appearance of the disease several of the plants in Lot 2 were completely dead and most of the others were badly diseased, while in Lot 1 a number of the plants showed signs of disease, but in none was it well advanced.

SUMMARY

This paper deals with (1) the relation of soil temperature to the occurrence of the wilt of tomato caused by *Fusarium lycopersici*, and (2) the effect of combinations of different soil and air temperatures on the disease.

When the fungus was exposed in Petri-dish cultures to temperatures ranging from 4° to 38° C., the optimum for its growth was found to be about 28° C., although abundant growth was secured all the way from 18° to 31° C. (See Plate VIII.)

In three experiments, soil temperature alone was varied. The plants were grown in soil which was first sterilized and then inoculated with *F. lycopersici*. The temperature of the soil was controlled to secure a range from 14° to 35° C. The period of growth at these temperatures was four to six weeks. The optimum soil temperature for the disease was found to be about 28° C., though at 31° it developed almost as virulently. At soil temperatures of 33° or above and of 21° or below, the disease was practically inhibited.

Two experiments were conducted in which both air and soil temperatures were controlled. For this work the air in three greenhouse compartments was maintained at temperatures designated as cool (about 17° C.), warm (about 27° C.), and hot (about 33° C.), respectively. Three of the Wisconsin soil-temperature tanks were placed in each house and regulated to hold the soil temperatures, likewise, at 17°, 27°, and 35° C., thus permitting nine combinations of air and soil temperatures.

Air temperature was found to be as effective in controlling the appearance of the disease as soil temperature.

In only two of the nine combinations of air and soil temperatures did the disease make a rapid development, these two combinations being warm air (27° C.) and warm soil (27° C.), and hot air (33° C.) and warm soil (27° C.).

If the soil was kept too cool (17° C.) or too warm (35° C.), the disease did not develop, even with optimum air temperature.

If the air was kept too cool (17° C.) and the soil temperature was optimum (27° C.) for the disease, heavy infection occurred in the root and extended up into the basal portion of the stem. The plants continued to grow thriftily, however, and there were no external symptoms of the disease.

The temperature conditions of soil and air most favorable for the disease, as determined in tanks, are a soil temperature of about 27°, and an air temperature, after the fungus has established itself in the stem, of about 28° C., with short periods when the temperature suddenly rises to an excessively high point (33° or 34° C.).

Evidence accumulated during the course of these experiments leads to the belief that the wilting and death of plants attacked by the *Fusarium* wilt disease is due not to mechanical plugging of the xylem bundles, but rather to toxic action.

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EXPLANATION OF PLATES

PLATE VIII

Petri-dish cultures of *Fusarium lycopersici* prepared by inoculating the center of each plate of potato agar with a single drop of a spore suspension of the organism. These were incubated for five days at a graduated series of temperatures ranging, as indicated, from 8° to 38° C. The highest temperature at which the fungus can develop, i.e., the maximum temperature, lies between 35° and 38° C., the optimum for its growth is about 28° C., and the minimum between 8° and 10° C.

PLATE IX

A. A plant inoculated and held at a warm temperature, favorable for the wilt (25°-30° C.), until the disease was well advanced, and then moved into a cool temperature (15°-20° C.) where the disease was checked. The stem on the right is the old main stem, all the leaves and the growing tip (x) having been killed by the wilt while the plant was still at the warm temperature; the growing tip, now dead and dry, may still be seen attached to this stem. The new growth, consisting of one shoot at the base of the stem and one higher up, was all formed after the plant had been exposed to the cool temperature. This new growth appeared perfectly healthy in every respect.

B. Representative plants taken from the temperature tanks in soil-temperature Experiment III. Note the prevalence of wilted plants between 22° and 31° C. and the healthy ones which grew at lower and higher temperatures. The optimum soil temperatures for the development of the disease, 28° and 31° C., stand out very conspicuously here.

C. Tops cut from control plants of the same experiment. They illustrate the vegetative development of uninoculated plants grown in sterilized uninoculated soil.

A comparison of photographs B and C shows the close correlation between the optimum temperature for the vegetative growth of the plant (22° to 31° C.) and the most pronounced development of the disease. Note also by comparison with Plate VIII that the same favorable temperatures hold for the development of the parasite.

PLATE X

Representative plants grown in inoculated soil for 20 days in the temperature tanks of soil-temperature Experiment III. These plants were grown under exactly the same conditions with the exception of the soil temperatures, which were held at 23°, 28°, 31°, and 33° C. respectively. These plants show more clearly than those in Plate IX, B, the stunted and wilted condition of the stems and foliage at 28° and 31° C. This is in sharp contrast to the absence of any signs of the disease at either the lower temperature (23°) or the higher (33°).

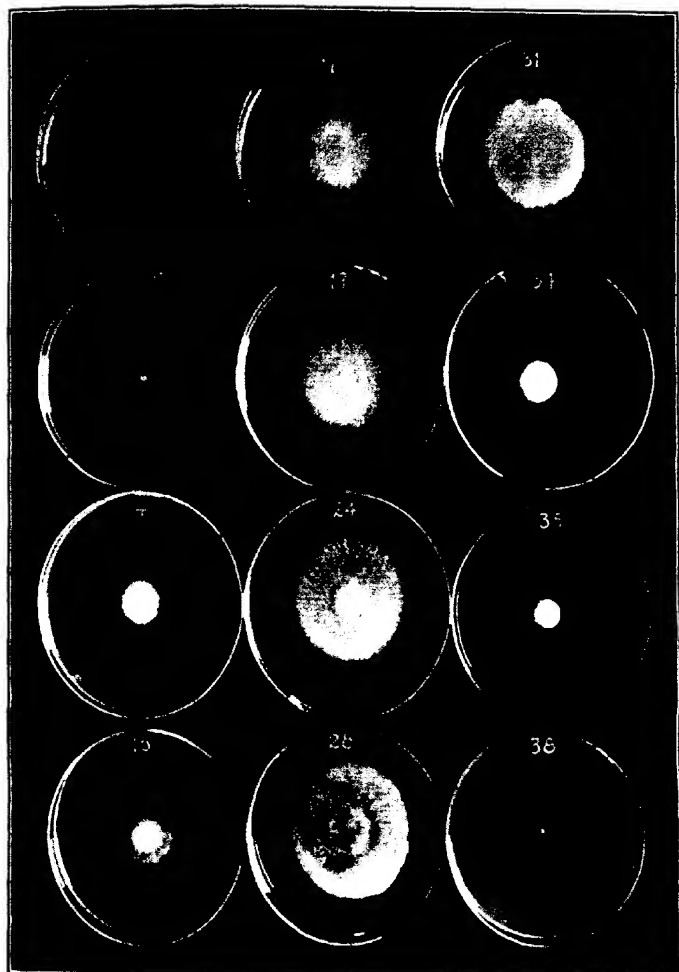
PLATE XI

The Effect of Different Combinations of Soil and Air Temperatures upon the
Tomato Wilt Disease

"A" is air temperature; "S," soil temperature

All plants alike were grown for one month in uniformly inoculated soil. Each pot is representative of a series, the different combinations of temperature conditions being shown in the labels.

Of the nine temperature combinations only two produced evident wilt symptoms, *viz.*, soil 27°, air 27°, and soil 27°, air 33° C. Especial attention is called to the middle plant in the upper row, grown in soil 27°, air 17° C. This soil temperature (27°) is the optimum for the disease: examination showed that the *Fusarium* had entered the roots, yet the disease was so inhibited by the low air temperature (17°) that the plants made a vigorous growth with no wilt. The plants at soil 17°, air 27° C., also deserve especial notice.



CLAYTON: TEMPERATURE AND FUSARIUM WILT



CLAYTON: TEMPERATURE AND FUSARIUM WILT



CLAYTON: TEMPERATURE AND FUSARIUM WILT



CLAYTON: TEMPERATURE AND FUSARIUM WILT

AMYLASE IN THE SPORES OF *RHIZOPUS TRITICI* AND *RHIZOPUS NIGRICANS*

L. L. HARTER AND J. L. WEIMER

(Received for publication April 28, 1922)

INTRODUCTION

The results obtained by a number of investigators have demonstrated that many fungi secrete enzymes, which diffuse out of the mycelium into the substrate, where certain cleavage products are formed.

However, Kopeloff and Kopeloff (3) seem to have been the first to study the enzymes of fungous spores. These workers found that the spores of *Aspergillus niger*, *A. sydowi*, and to a lesser extent *Penicillium expansum* and *A. flavus* contain an enzyme which hydrolyzes cane sugar. Their results show that the deterioration of cane sugar may depend, in part at least, on the action of the spores of some of the mold fungi. The enzymic activity of the spores, previously killed by heating for 30 minutes at 63° C., was determined by suspending them in cane-sugar solution of known strength and determining the cleavage products by polarization. The rate of hydrolysis of the cane sugar was found to be correlated with the number of spores present in the system.

It is the purpose of this paper to record the data obtained relative to the occurrence of amylase in the spores of *Rhizopus nigricans* Ehrhb. and *R. tritici* Saito.

METHODS OF EXPERIMENTATION

Since the mycelium of *Rhizopus tritici* has been shown to produce an amylase (2), it was imperative that the spores be completely separated from it before this enzyme could be studied. Preliminary experiments showed that spores were produced abundantly and that they could be readily separated from the mycelium if the fungi were grown on sweet-potato decoction; hence this medium was employed. About 750 cc. of the solution were used in 2-liter Erlenmeyer flasks. On this medium a luxuriant growth is made in from 5 to 10 days at temperatures ranging from 22° to 35° C. The spores, although abundantly produced in about 5 days, separated from the mycelium much more readily after about two weeks' growth; hence cultures of this age were used. At the end of the growth period, the fungus, which formed a thick felt floating for the most part on the surface of the medium, was carefully removed from the flasks. The bottom of the felt was held under a stream of running water in order to remove the culture solution. It was then floated top down in a dish of distilled water

and carefully agitated to remove the spores. By this method a considerable portion though not all of the spores were removed. To insure a complete separation of the spores from the mycelium which might have washed off into the water, this spore suspension was filtered through two thicknesses of a good quality of muslin, of about 20 threads to the centimeter. Most of the spores passed through the muslin while the mycelium was held in the cloth. At this time a microscopic examination of some of the filtrate was made to determine if any of the mycelium passed through the muslin. In case any of the mycelium was found mixed with the spores, the entire suspension was discarded. It is possible that some short mycelial threads escaped notice, but the writers feel certain that these were not sufficiently numerous to account for more than a very small part of the hydrolysis of the starch which actually took place. The spores were collected on a no. 2 Whatman chemically prepared filter paper, treated with a large volume of acetone for 10 minutes, and collected on a tarred filter paper (no. 2 Whatman). They were then immersed in ether for 3 minutes and dried to constant weight at a temperature of about 30° C. in a desiccator over calcium chloride. No germination of the spores could be obtained after treatment with the acetone and ether.

The hydrolysis of the starch paste was carried out in 150-cc. pyrex flasks for 18 hours. The temperature differed somewhat with the different experiments. The amount of spore material varied somewhat, but 100 cc. of starch paste were always used. About 3 cc. of toluol were used as an antiseptic. At the end of the digestion period the system was heated for 10 minutes in the autoclave to inactivate the enzyme and was then filtered through a good grade of filter paper. The reducing sugars formed were determined volumetrically according to the method of Clark (1).

Only in a few preliminary experiments was an attempt made to separate the spores from the filter paper before they were used for the hydrolysis of starch. This process was attended by many difficulties and was finally abandoned. In all subsequent experiments the filter paper on which the spores were caught was included with the spores in the system. Before the starch paste was added the spores were suspended for 3 hours in 20 cc. of distilled water. Tests were made to determine if reducing sugars could be obtained from the filter paper, but only in one case, and that within the limits of experimental error, were any found. No reducing sugars were obtained from the starch when it was suspended in sterile distilled water.

EXPERIMENTAL DATA

Experiment 1

The spores were obtained from a culture of *Rhizopus tritici* incubated at a temperature of from 22° to 25° C. The weight of the spores used in this experiment was not determined. Most of the spores were washed

from the filter paper in 30 cc. of water and extracted for three hours at 25°. After thorough shaking in order to obtain as uniform a distribution of the spores as possible, 10 cc. were pipetted off into each of two flasks, thus making three flasks each containing an equal amount of the spore suspension. To one flask (A) were added 40 cc. of a 0.5 percent starch-paste solution. A like amount of starch-paste solution was placed in the second flask (B), which was then steamed for 10 minutes to inactivate the enzyme. To the third flask (C) 40 cc. of sterile distilled water were added. Hydrolysis was carried out for 18 hours at 38°. Results (average of several tests) expressed in milligrams of reducing sugars per 100 cc. of solution were obtained as follows: A, 105.3; B, 0; C, 0.

Experiment 2

Organism, *Rhizopus tritici*. The purpose of this experiment was to show that the enzyme is produced in the spores at any temperature at which the fungus will grow. The exact quantity of spores used in these experiments was not determined. Cultures of the organism were grown at three temperatures, 9°, 30°, and 40° C. At 9° no fruiting took place, but at temperatures of 30° and 40° spores were abundantly produced. The spores were extracted for 3 hours in 10 cc. of water. Digestion of starch (50 cc. of a 0.5 percent starch-paste solution) was carried out for 18 hours at 38°, with the following results expressed in milligrams of reducing sugars per 100 cc. of solution: 30°, 163.8; 40°, 256.1. There were more reducing sugars formed by the spores from a culture grown at 40° than from one grown at 30°. However, this does not necessarily mean that spores produced at the higher temperature have a greater hydrolyzing power, since there may have been more spores present in the system. It does mean, nevertheless, that spores produced at such a temperature are capable of hydrolyzing starch to sugar.

Experiment 3

Experiment 3 represents the average of a number of parallel tests in which the amounts of the reducing sugars formed are expressed in milligrams per 100 cc. of solution. The exact weight of the spores was determined in each case, and the amount of starch hydrolyzed was calculated for 0.25 gram of spores.

TABLE 1. Amount of reducing sugars formed in a solution of starch paste by the spores of *Rhizopus tritici* and *R. nigricans*, expressed in milligrams per 100 cc. of solution

Temperature (° C.)	<i>Rhizopus tritici</i>	<i>Rhizopus nigricans</i>
22 to 25.....	335.35	103.23
30.....	—	215.00
38.....	189.49	—

The spores were prepared according to the method already described. They were extracted in 20 cc. of sterile distilled water for 3 hours. After the addition of 80 cc. of a 0.5 percent starch-paste solution, digestion was carried out for 18 hours at 38° C. The reducing sugars formed in the system

were determined in 10-cc. portions in duplicate. The results of these experiments are given in table 1. The spores were obtained from cultures grown at the temperatures indicated in the table.

It will be seen that the range of temperatures employed is somewhat limited. The object of these experiments was primarily to prove that a starch-hydrolyzing enzyme was contained in the spores of *Rhizopus tritici* and *R. nigricans*. It was previously shown by Harter (2) that the mycelium produced amylase when grown at temperatures ranging from 9° to 40° C., but not uniformly so. The enzyme was most active when the fungus was grown at 9° and least active when grown at 40°. Both of these fungi will grow and produce mycelium over a considerably wider range of temperatures than those at which spores are produced. As a matter of fact, *R. tritici* will grow at a much higher temperature than *R. nigricans*, while the latter will thrive better at a lower temperature than the former. The range of temperatures at which spores are produced is therefore limited in both organisms. *R. tritici* produced spores abundantly at temperatures ranging from approximately 20° to 38°, and *R. nigricans* from about 16° to 30°. Above and below these temperatures the quantity of spores was so small as to make the determination of the hydrolysis of the starch out of the question.

Within the limits of these experiments the results indicate that amylase is contained in the spores at any temperature at which they are produced. The same was also found true with respect to the mycelium. The data at hand are not sufficient to justify the conclusion that the amount of amylase contained in the spores is correlated in any way with the temperature at which the organism was grown.

Comparative tests of the hydrolyzing power of the spores and mycelium have shown that the enzyme of the latter is more active than that of the former, when compared on the basis of unit weight. This holds true when the comparison is made between spores and mycelium grown at the same temperature.

SUMMARY

1. An enzyme capable of hydrolyzing Irish-potato starch paste to reducing sugars is produced in the spores of *Rhizopus tritici* and *R. nigricans*.
2. Within the limits of these experiments the enzyme is produced at any temperature at which spores are produced.

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OBSERVATIONS ON THE CAUSES OF GREGARIOUS FLOWERING IN PLANTS¹

WILLIAM SEIFRIZ

(Received for publication May 11, 1922)

One of the most interesting and fundamental of biological problems is that of the extent to which the life processes of an organism are influenced by the external environment. In the past, biologists have been quite content to rest secure in the belief that the most deep-seated characters in organisms are developed in the individual and transmitted from one generation to another little influenced, and certainly not determined, by the external environment of the organism. That so fundamental a character as paired eyes in vertebrates could in any great degree be influenced by a change in external environment was hardly conceivable until Stockard showed that if the eggs of the fish *Fundulus* are placed in sea water to which a little magnesium chloride has been added they develop into embryos with one medium cyclopean eye.

Equally interesting to the botanist have been the experiments of Garner and Allard (6), who were able by controlling the time of exposure of a plant to light greatly to lower or increase the age at which the plant reaches sexual maturity. Thus, the field aster, which commonly requires four months (May to September) to reach sexual maturity, was made, by decreasing the time of exposure to daylight, to bear flowers within a month after germination (by June 18). Still more remarkable is the fact that these same plants, instead of completing their life cycle by dying after flowering, as they would have done in the field, developed new axillary branches (on being restored to normal light exposure) and flowered a second time in September.

It is thus evident that certain characters of a deep-seated and fundamental nature which heretofore have been regarded as immutable, are relatively unstable and respond readily to changes in the external environment. It is, consequently, not surprising that some biologists hold that all "characters are of the nature of responses to environment" (7, p. 530), and that "every life process must to some degree be dependent upon the external world" (15, p. 285).

While it is difficult to deny the truth of these statements in the face of the remarkable experiments which have been performed, yet one wonders how far such a theory will carry us. We hesitate to admit that the external environment is in any way responsible for the fact that a pine seed develops

¹Contribution from the Oakes Botanical Laboratory.

into a pine. We may even go further than this and maintain that the spreading form of the common poplar (*Populus nigra*) and the tall slender form of the Lombardy poplar (*P. nigra* var. *italica*) must, since the latter arose as a mutation of the former and since both forms breed true in the same environment, be due to the special nature of the protoplasm of the two trees and not to environmental factors. Those who hold the extreme view that "all characters are innate, acquired, germinal, somatic, and inheritable in exactly the same sense and degree" (7, p. 596) will find some difficulty in explaining the origin of fluctuations and mutations in certain individuals of a species when other individuals of the same species in the same environment remain true to type.

That external environment plays an important if not an inseparable part in inheritance and development is a fact of far-reaching significance. The problem of the biologist is to determine *to what extent* the heritable disposition of plants is susceptible to influence by the external environment.

In a study of this sort one must guard against concluding that an environmental factor is the *cause* of a vital phenomenon because it has been found to influence the phenomenon. Thus, from Garner and Allard's work we may conclude that length of exposure to light determines the *time* of flowering in certain plants, but we are not justified in regarding this environmental factor as the *cause* of flowering. The act of attaining sexual maturity is innate in all organisms, just as is senility. The *exact point* in the life cycle at which the individual shall become sexually mature is in some plants evidently sensible to external influence; *i.e.*, it is capable of being shoved this way or that by the environment.

The problem of determining the environmental factors which influence the flowering of plants assumes a special interest when considered in reference to those plants which flower only after a period of many years of purely vegetative growth. Particularly interesting does the problem become when applied to those plants in which all the individuals of a species throughout a given region attain sexual maturity simultaneously. The bamboos (many of them) belong to this category.

DROUGHT AS A CAUSE OF GREGARIOUS FLOWERING IN BAMBOOS AND PALMS

One of the oldest theories of the cause of gregarious flowering in bamboos is that this simultaneous anthesis is occasioned by drought (18, p. 251). This theory is still held by many biologists. Some (8, 16) have advanced another hypothesis, namely, that periodic flowering in bamboos is the result of a depletion of nourishment. Both theories are opposed to that which would attribute this sex phenomenon to a heritable factor in the plant.

The chief criticism to be directed against such theories is that their supporters have failed to look further than the particular group of plants and the special set of environmental factors under observation.

That bamboos have flowered gregariously in India immediately after a drought is not to be doubted. Droughts are of such frequent occurrence in India that it would be surprising if they did not occasionally coincide with the flowering of bamboo forests. It is also possible that severe dry weather may have some *slight* influence on the *exact time* of flowering. When many individuals of a species flower simultaneously immediately after a drought, scientist as well as layman is likely to associate the two phenomena. The occurrence of each phenomenon separately passes unnoticed. But even if our data should warrant the conclusion that the gregarious flowering of a particular species is occasioned by drought in a certain locality, what are we to do with the fact that the same species flowers in another locality where there is no drought? *Bambusa arundinacea*, for example, flowers not only in India, where it is subjected annually to a severe dry season and occasionally to a drought, but also in Buitenzorg, Java, where dry seasons are practically unknown. Then, too, we have the interesting fact that other species of bamboo flower gregariously following an unusually *wet* period, as did, for example, the climbing bamboo, *Chusquea abietifolia*, in Jamaica in 1918 (20). Still other bamboos show no periodicity at all and flower sporadically without any apparent relation to climate. This is true of the Philippine bamboos in general, among which no case of simultaneous flowering of many individuals is known, although these bamboos have been under scientific observation for nineteen years.²

So far as I have knowledge, no one has actually investigated the rain-fall data of the country where bamboos flower gregariously in order to ascertain, first, whether or not the dry season of the particular year in question was one of sufficient severity to warrant its being regarded as the direct cause of the gregarious flowering of the bamboos; and, second, whether or not, in case a drought did precede the particular flowering period under investigation, other flowering periods of the same species of bamboos in that country (and in other countries) were also preceded by droughts.

The meteorological conditions prevailing in India are so extreme that one must be thoroughly aware of them in order to investigate intelligently a question such as that under consideration. The greater part of India is almost rainless for about seven months of the year. It is not an uncommon occurrence for no rain whatever to fall at certain stations during eight months of the year. The absence of rain during such a normal dry season cannot, of course, in any sense be regarded as a drought. What rain does fall during the dry season (averaging 0.2 to 0.3 of an inch a month) is of little consequence to plant life. Vegetation depends solely on the monsoons, which occur from June to September in Hyderabad and the Central Provinces, for example, and from October to December in Southeast

² Dr. E. D. Merrill, Director of the Philippine Bureau of Science, has kindly given me

Madras. If the monsoon rains fail, severe consequences follow. This is what happened in 1899 and 1900. There was a phenomenal failure of the rains over a large part of India during the 1899 monsoon (June to September). As a consequence, a disastrous drought prevailed in the early months of 1900. Both immediately *before* and some time after this severe drought large bamboo forests flowered in northern India.

In the Indian Forester for 1899 there appears the following note (10, p. 178):

The flowering of *Bambusa arundinacea* is reported . . . to be general this year in the Angul Division³ of the Bengal Presidency.

The drought of 1899-1900 above referred to could have had no effect on the life of these bamboo forests, since the bamboos were in flower (in April) before the failure of the monsoon rains (June to September), the disastrous consequences of which could not have been felt by plants until early 1900. In 1898, the year preceding the flowering of the bamboos in the Angul District, the monsoon was normal—at Angul (41.29 inches) slightly below the average (49.34 inches), and at Bissipara (58.49 inches) somewhat above the average (55.07 inches). For the four preceding years (1894-1897) the total annual rainfall in the Angul District was either just at the average or considerably above average—never below. We cannot, therefore, accredit the general flowering of *Bambusa arundinacea* in the Angul District of India in 1899 to a drought.

In the Indian Forester for 1901 (11, p. 126) is reported

The flowering on a large scale of the ordinary bamboo (*Dendrocalamus strictus*). The area over which the flowering extends is estimated at 1200 square miles, and in this area, although a few clumps here and there have escaped, the phenomenon is universal.

The flowering occurred in the Chanda District of the Central Provinces of India. The rainfall data for 1900 from sixty-seven observation points in the Central Provinces indicate that the monsoon of that year was all that could be desired. For example, the total annual rainfall for forty-five of the sixty-seven stations was, in 1900, above the annual normal (in several instances nearly double the average annual precipitation).

It would hardly seem necessary to go further back than the favorable monsoon of 1900 in our investigation of meteorological conditions and of their bearing on the flowering of the bamboo forests in the Central Provinces of India in 1901. This statement is based on the assumption that the visible effect of a climatic influence which is potent enough to affect the physiological state of plants is likely to become evident within a year after the climatic factor came into existence. To what extent such an assumption is justifiable is an open question. Brandis (1, p. 14) believes "that such stimulating conditions must act upon the plant at least a year before the flowering actually takes place." Yet in the same article he refers to

³ The official meteorological designation of the "Division" in which the Angul "District" occurs is the "Chota Nagpur Division" of the Bengal Presidency.

the observations of Kurz in Burma where an unexpectedly large number of bamboos were collected in flower "*during* [italics mine, W. S.] the two dry seasons of 1868 and 1869." Brandis further quotes Kurz as stating "that in the Calcutta Botanic Garden there never had been so many species in flower as in 1874, which was a year of great drought" (10, p. 14). It will be noticed that the abundant flowering of the bamboos in the Calcutta Garden occurred in 1874, *i.e.*, during the year of great drought, and that the supposed stimulating conditions did not act upon the plant "at least a year before the flowering" actually took place, as Brandis elsewhere maintains must be true.

To go further back than the favorable monsoon of 1900, *i.e.*, to go back more than a year previous to the flowering of the bamboos in 1901, involves the question whether or not meteorological conditions, occurring more than a year before the appearance of the vital process which they are supposed to initiate, can be taken into consideration. It seems hardly likely that bamboo plants which flowered in early 1901 and whose flowering was preceded by a favorable rainy season in the mid-year of 1900 should have flowered as a result of a drought in early 1900. Not only Kurz, whom Brandis quotes, but others who have ascribed the gregarious flowering of bamboos to drought, have spoken of the flowering as occurring in *times* of drought.

Owing to the severity of the drought of 1899-1900, we cannot altogether ignore the possibility of the unfavorable climatic condition having initiated in the bamboos of northern India a physiological process which did not become externally evident until a year and a half later (in the simultaneous flowering of the bamboo forests in 1901). Once the marked change in the physiological state of the plants was initiated, a subsequent favorable climatic condition (the monsoon of 1900) would be of no effect.

The question cannot be conclusively answered. It is possible that the extreme drought of 1899-1900 of the Central Provinces of India had a telling effect on the bamboos of that region. But that the drought was the *cause* of the simultaneous flowering of the bamboos is not, in the face of other data, a possible deduction. The most that can be said is that when bamboos are near their time of reproduction an unusually dry season may have the effect of accelerating the formation of flower buds.

Whatever our decision regarding the possible effect of the drought of 1899-1900 on the general flowering of *Dendrocalamus strictus* in the Central Provinces of India in 1901, we have the definite fact that *Bambusa arundinacea* flowered gregariously in India in 1899 in the absence of a drought for at least five years previous to the flowering.

Another interesting bit of evidence against the theory that drought is the cause of gregarious flowering in bamboos of long life cycle is to be found in the behavior of an immense bamboo forest region in Burma. The bamboo in this case is of another species (*Bambusa polymorpha*) than the two (*Dendrocalamus arundinacea* and *D. strictus*) we have just been considering.

The sexual cycles of *D. arundinacea* and *D. strictus* are about thirty-two years in length. *Bambusa polymorpha* is known to have a very long life period. I know of no authentic record of two successive flowering dates.

In the Indian Forester for 1903 appears the statement that "the last recorded flowering of the Kyathaung was . . . in 1853" (13, p. 244). The flowering of this bamboo, *Bambusa polymorpha*, was expected to recur shortly after 1883 on the general belief that the life of bamboos is about thirty years. The flowering of *B. polymorpha* in the forests of Burma has not yet occurred. Certain "signs" of an expected flowering have from time to time been seen. These signs refer to the well-known habit which bamboos have of producing no new shoots in the year of flowering.

The bamboo forests of *B. polymorpha* in Burma may be reckoned by hundreds if not by thousands of square miles. In this extensive region of bamboos there have been, since 1883, one or two false alarms of gregarious flowering when a clump or two has blossomed. In 1918 and 1919 an area of several hundred acres in two or three distinct but neighboring blocks in the Tharrawaddy Division flowered gregariously.⁴ (The plants of *B. polymorpha* were at this time sixty-five years of age.) This was thought to be the forerunner of a general flowering, since the flowering of odd clumps is considered to be an indication that the flowering of the whole area is imminent. But so far no general flowering has taken place.

During their sixty-eight years of existence the bamboos of these forests have endured many droughts which apparently have had no effect whatsoever on the sexual maturity of the plants. For at least the latter half of their life the bamboos must certainly have been mature enough to respond to an external stimulus, if this stimulus is of such a nature as to exercise any prominent influence on the sexual life of the plants.

We have so far seen, first, that bamboos of long and rhythmic life cycles reach sexual maturity when experiencing only the normal annual dry season of the tropics; and second, that other bamboos of long periodicity have for sixty-eight years failed to attain sexual maturity even though they have experienced many seasons of both normal dry weather and drought. There now remain to be considered those instances in which flowering takes place in the entire absence not only of drought but even of a typical tropical annual dry season.

The most striking instance of this is the behavior of the bamboos at Buitenzorg, Java, where droughts are unknown and dry seasons are few and far between. Before passing on to the Buitenzorg data it will be interesting to consider in more detail the similar case of the climbing bamboo, *Chusquea abietifolia*, already referred to. This bamboo, a slender scandent form, occurs in great abundance throughout the mountains of Jamaica. The high altitude regions of Jamaica receive abundant moisture

⁴ I am indebted to H. R. Blanford, Esq., O. B. E., Government silviculturist at Maymyo, Burma, for this information.

at all times of the year. Some few arid localities occur, but they are small and infrequent above 4000 feet. Relatively dry weather may come occasionally at high altitudes, but never a drought above 5000 feet, the elevation at which *Chusquea* grows. *Chusquea abietifolia* flowered gregariously in the mountains of Jamaica in 1918 (20). Over an area ten miles in length (investigated by the writer) the trails were, in 1919, lined with dead tangled masses of this climbing bamboo. The two years immediately preceding the flowering of *Chusquea* were (at Cinchona) unusually moist ones. It is interesting to note that specimens of *Chusquea abietifolia* sent to Kew, England, in 1884, a year prior to the last previous flowering of the plants (in 1885, the life cycle of *Chusquea* being thirty-three years), flowered simultaneously with the plants in Jamaica.

The behavior of *Chusquea* in Jamaica stands in further opposition to the belief that lack of moisture may cause flowering in bamboos in that it does not support the statement of Brandis that "there are indications, that in dry stony places . . . bamboos flower earlier and more abundantly" (1, p. 662). It was in just such places that the only green living specimens of *Chusquea abietifolia* were found in the mountains of Jamaica. On an exposed hot and dry spur, sparsely covered by a typically xerophytic vegetation, were growing a quantity of old, green, and thriving specimens of the climbing bamboo. Immediately below this dry spur on which living old plants of *Chusquea* were growing, there is a moist, cool ravine. Here no adult living specimens were found, but there existed instead the condition prevailing generally throughout the mountains: old plants were dead and growing seedlings were abundant. The old living specimens on the arid spur above were not in fruit. Flowering had not taken place earlier, as Brandis suggests, but, on the contrary, had been delayed. Possibly the climbing bamboo had in this more arid region assumed an altered life cycle.

In comparing the behavior of the bamboos at Buitenzorg, Java, where droughts are unknown and dry seasons are infrequent, with the behavior of the bamboos in India and Ceylon, where dry seasons of several months come annually and droughts occur frequently, it will be well to consider with the bamboos the equally instructive case of the talipot palm, *Corypha umbraculifera*, which, like some bamboos, has a long vegetative period at the expiration of which the palm flowers and dies. Ordinarily *Corypha umbraculifera* does not flower gregariously nor at a fixed age, as do certain bamboos. Consequently, when many specimens of the talipot palm do flower simultaneously, one is likely to suspect the presence of some external factor which has aroused the palms to sexual activity.

The most remarkable case of simultaneous flowering of plants of which I know is that which recently occurred at Peradeniya, Ceylon. In the annual report for 1918 of the Director of the Royal Botanic Gardens at Peradeniya (4) there appear the following three notes:

Seven out of the sixteen talipot palms (*Corypha umbraculifera*) forming the avenue,

started by seed sown *in situ* in 1881, commenced to flower in June, and continued in blossom until the end of the year, being at their best in October–November [Pl. XII, fig. 1].

Bauhinia anguina, a very large woody climber with peculiar alternately compressed chain-like stems, has this year fruited for the first time on record at Peradeniya. Trimen, in his *Flora*, states: "Very rare, flowers and fruit not seen."

The flowering of the giant bamboo (*Dendrocalamus giganteus*) is not now the rare event in Ceylon it used to be. Nine clumps produced flowering stems during the early dry months of the year. . . . None of these clumps have died. Eleven clumps of the "male bamboo" (*Dendrocalamus strictus*) also flowered profusely early in the year. Of these, five clumps have died in consequence.

To this is to be added the interesting fact that at the same time that the talipot palms were blossoming in the Gardens there were counted from one observation point elsewhere in Ceylon two hundred talipot palms in flower. So extraordinary a concurrence of the profuse flowering of four species of plants, all of whose life cycles are very long—in the case of the two bamboos about thirty-two years, of the talipot palm nearly forty years, and of the liane *Bauhinia* so long that there is no record of it—is indeed an event that forces one to search for some possible environmental factor which might be responsible.

The annual dry season of 1918 was in Ceylon longer than usual, sufficiently long to be locally termed a drought. The remarkable flowering of so many talipot palms throughout Ceylon and the simultaneous flowering of three other species of plants of long sexual periodicity was attributed to this drought.

Three objections can be raised against such a deduction. First, the drought of 1918 was a relatively mild one. The total precipitation for the four months (January to April) of the dry season was, to be sure, below the average for this time of year (12.9 inches in 1918 as compared with a normal of 17.05 inches for these four months, all averages being based on twenty years' records from 1901 to 1920); yet the difference is not very great. Furthermore, if we review the records of the years immediately preceding, we see that the dry season of the second year before the flowering of the talipot palms and bamboos in the Gardens was also below the average; not quite so low for the four months of the dry season as in 1918, but much lower for January, when but 1.0 inch of rain fell (in 1916) as compared with 5.23 inches in 1918. And in February, 1916, there was but 0.03 inch of precipitation. One would expect these two very dry months of 1916 to have a more telling effect on plant life than the dry season of 1918. If we go further back we find that there was a drought in 1903, and again in 1905, of much greater severity than that of 1918, especially the latter one (1905) when the total rainfall for the four months January to April was the lowest on record for twenty years (1901 to 1920), namely, 4.9 inches or nearly one third that of 1918. In 1911 there occurred at Peradeniya a drought which, because of its duration, was more severe than any so far mentioned. In this year there fell during the *five* months of January to May but 12.78

inches of rain as compared with a normal average of 21.95 inches for this period. Especially trying must this drought have been on plant growth in view of the fact that the precipitation in April, which usually ends the normal dry season, was less than half the average, while in May there fell but 0.75 inch of rain as compared with a normal precipitation for this month of nearly five inches (4.90). In 1911 the talipot palms were but seven years younger than in 1918, *i.e.*, they were thirty years old, not too young to respond sexually to an external stimulus of some force.

Through all these droughts the six talipots, with others in the Peradeniya Gardens and hundreds throughout Ceylon, the twenty clumps of bamboo, and the liane *Bauhinia*, grew on without flowering. It is therefore hardly likely that the relatively mild drought of 1918 had any influence on the flowering of these plants.

The second objection to be raised against the conclusion that drought caused the flowering of the plants in the Peradeniya Gardens is that nine out of the sixteen talipot palms did *not* flower, and these nine were of the same age and had been growing under the same conditions as the seven which did flower. Obviously, if flowering was the direct result of drought or of any other climatic factor, the seven palms which were affected must have been in such a physiological state as to be susceptible to the influence while the other nine palms were not in such a state. That is, if drought is a factor it is a secondary one, the physiological condition of the plant being the primary determining factor.

If we conclude that the ultimate cause of the time of attaining sexual maturity lies in the hereditary disposition of the plant, the interesting question arises, Why did seven of the talipots flower and nine not, since all in the avenue were of the same age? We can only attribute this difference in behavior to individual differences in the germ plasm, concerning the causes of which we know nothing. The age at which *Corypha umbraculifera* reaches sexual maturity is not the same in all individuals.

The final and most convincing evidence against the hypothesis that drought is the *direct* cause of flowering, or even a factor of any great significance in the flowering of certain palms and bamboos, is the behavior of another talipot at Peradeniya and of a talipot and the bamboos at Buitenzorg. The talipot in question at Peradeniya is one which flowered some years ago, in 1903 (fig. 2). For four years (1899-1902) previous to the flowering of this palm at Peradeniya the average annual rainfall was, in each of these four years, above the normal average. In 1902, the year immediately preceding the flowering, the total annual rainfall was approximately one third above the normal average. It is quite evident that the flowering of this talipot can in no way be attributed to drought.

When the many talipots in Ceylon were blossoming in 1918, the only old specimen of this palm in the gardens at Buitenzorg was also in flower (fig. 3). At Buitenzorg there is no such thing as drought. The writer was

there during the so-called dry season and rain fell in torrents nearly every afternoon. While the dry season characteristic of Java as a whole is sometimes more or less evident at Buitenzorg, it never assumes the proportions of a drought. The avenue of talipot palms at Peradeniya and others on the island of Ceylon which flowered in 1918 had been subjected to a prolonged dry season immediately preceding the time of flowering and to several severe droughts during their thirty-eight years of existence. The *Corypha* at Buitenzorg, on the other hand, had been drenched in rain nearly every day of its life; yet on both islands the palms flowered in the same year.

It would be interesting to know if the flowering of the Buitenzorg talipot commenced in the same month, June, 1918, as did that of the Ceylon palms. One would be inclined in such a case to suspect the presence of some meteorological influence of wide distribution, if one is willing to place any faith in an external stimulus as an influencing factor of even secondary importance. That the palms in Ceylon and at Buitenzorg did flower at very nearly the same time of year is evident from the Peradeniya data and from my observations in Java. (Unfortunately no records are kept of the date of flowering of plants in the Buitenzorg Gardens.) The palms at Peradeniya flowered in June, 1918, fruited during the latter part of 1919 and early in 1920, and died in 1921. The *Corypha* at Buitenzorg had just dropped its fruit when I first saw it in August, 1920 (fig. 3).

What is true of the talipot palms at Peradeniya and at Buitenzorg is also true of the bamboos at the two gardens. At the time of my stay in Buitenzorg, seven species, out of a total of twenty-four in the Gardens, were in flower. One of these species was *Dendrocalamus giganteus*, which was in heavy flower. The species is the same as that of one of the bamboos which flowered at Peradeniya in 1918. This *D. giganteus* and the six other flowering species of bamboo at Buitenzorg had not been subjected to a drought nor even to a characteristic tropical dry season.

We are, it seems to me, forced to conclude that the ultimate cause of gregarious flowering in bamboos of long life cycle, in particular *Chusquea abietifolia*, *Bambusa arundinacea*, *B. polymorpha*, *Dendrocalamus giganteus*, and *D. strictus*, and in the talipot palm, *Corypha umbraculifera*, is not drought. If drought is at all an influencing factor, then its effect must be relatively slight. While the simultaneous attaining of sexual maturity of three widely differing genera of plants, all of whose life periods are of great length, is an event of such unusual occurrence that one is inclined to wonder if there might not be an external stimulus which is responsible, yet if some such environmental cause does exist we are totally ignorant of what it may be.

THE RHYTHM IN THE SEXUAL CYCLE OF BAMBOOS

Evidence of a different kind, which stands in opposition to the assumption that the attaining of sexual maturity of bamboos of long life cycle is

greatly influenced by environmental factors, are the observations made in India which have twice established the cycle of *Bambusa arundinacea* to be exactly thirty-two years. This bamboo flowered in Cisgangetic India (the west coast) in 1804, 1836, and 1868 (18, p. 251). It is of interest to note here that the life cycle of *Chusquea abietifolia*, quite a different type of bamboo, is also about thirty-two years (recorded as thirty-three years) (20).

Less definite, and tending more strongly to support the supposition that the *exact time* of flowering may possibly be somewhat advanced or delayed by external factors, are the interesting data of Kawamura who states that the flowering of *Phyllostachys puberula* has been recorded in old manuscripts of China and Japan as occurring in the following years: 292, 813, 931, 1114, 1247, 1666, 1786, 1848, and 1908 (14). It will be noted that most of the intervals between these dates are either about sixty or multiples of sixty years.

One can, of course, fall back upon the assumption that climatic cycles of rhythmic periodicity also occur and that these determine the regularity of the sexual functioning of bamboos. While certain meteorological phenomena take place rhythmically, e.g., the annual seasonal cycle, it is as yet by no means well established that climatic changes of many years' duration are periodic, and there is little evidence that droughts occur rhythmically.

The evidence so far presented is overwhelmingly against the belief that drought is either the cause of gregarious flowering or that it has any marked influence on reproduction in bamboos. That climate may possibly exert some slight effect on flowering is not, however, to be emphatically denied. While there is little and only indirect evidence in support of this possibility, it is quite conceivable that as a plant of long sexual cycle nears its time of reproduction, unfavorable conditions may hasten the sexual process somewhat. Such a supposition would assist in explaining so remarkable a concurrence of simultaneous flowering as occurred in the Peradeniya Gardens in 1918, though here we must presuppose some climatic factor other than drought.

Depletion of nourishment and injury are two other factors external to the plant which have been advanced as causes of flowering in bamboos. One of these, injury, cannot be regarded as a natural cause of flowering. It has, therefore, no direct bearing on our problem, but it is of interest, and we shall consider it.

DEPLETION OF NOURISHMENT AS A CAUSE OF FLOWERING IN BAMBOOS

The Japanese worker Hori (8) is of the opinion that flowering in bamboos is a "physiological disease." This opinion is in contradiction to that of his fellow countryman Kawamura, who attributes the cause of flowering to the hereditary disposition of bamboos.² (The observations of Kawamura

² I am indebted to Professor Manabu Miyoshi for calling my attention to work done in Japan on the flowering of bamboos.

will be considered later.) Hori regards flowering in bamboos as a result of an increase in sugar content of the sap due to the inability of the plant to attain the necessary salts for nourishment owing to the dryness of the soil. While Dr. Hori's interesting observations (of which he has kindly given me a *résumé*) tend to support his theory, they cannot be regarded as generally applicable, since, as we have seen, bamboos flower even though profusely watered, and also fail to flower though subjected to repeated seasons of drought.

Macmillan (16) is also of the opinion that a depletion of nourishment is the cause of flowering in some bamboos. He states (p. 125):

It would thus seem as if the exhaustion of nutriment rather than an infectious influence were responsible for the more or less simultaneous flowering of the Giant Bamboo. The vigorous growth of the plant is such that it cannot go on growing and extending indefinitely. The enormous demands it makes on the soil can be realized by any one who has seen the "ruins" of an old clump, the huge crevices and upheavals formed by the elevated stumps as if the result of an earthquake.

Macmillan's description of the elevated base of an old bamboo clump is very graphic. But on such a mound of stumps measuring fully eight feet in diameter and three feet in height I have seen healthy culms growing as luxuriantly as those of any bamboo clump in the Buitenzorg Gardens.

Macmillan reports the continuation of the vegetative growth of two clumps of *Dendrocalamus giganteus* as a result of increased nourishment. It seems that two of the flowering clumps at Peradeniya, having regained a more vigorous condition, "gave up blossoming entirely, presumably because their circumference had struck richer soil" (16, p. 125).

This instance at Peradeniya is especially interesting because the bamboo in question happens to be of the same species as a young plant recently growing in the Buitenzorg Gardens, which was transplanted from an old clump and thus given an opportunity to regain a more vigorous condition by striking new soil. But it refused the opportunity and soon followed in the path of the parent plant.

There had been growing for many years in the Buitenzorg Gardens a magnificent clump of *Dendrocalamus giganteus* remembered for its size and beauty by all the older workers of the Lands Plantentuin. In 1918 this entire clump of bamboos flowered and died. Not wishing to lose the last specimen of so fine a bamboo (seeds are not produced), the director of the gardens had a few culms, which were still in healthy condition, removed from the parent clump as soon as the latter commenced to flower. It was hoped that these transplanted culms would continue their vegetative growth without flowering. Such was not the case, however. One of the transplanted clumps soon flowered and died. The second clump lived scarcely more than a year after transplanting, when it too flowered and died. I saw this small plant when the long pendent blooms were still hanging to the then nearly dead culms (fig. 4). New and richer soil did not cause this

Dendrocalamus giganteus to give up flowering and continue its purely vegetative growth. Similar observations must have been made by Brandis, since he writes that "offsets taken from a clump some time before it flowers come into flower at the same time as the parent clump" (1, p. 662).

The hypothesis of depletion of nourishment as the cause of flowering in bamboos could never be applied to those bamboos which flower gregariously. It is quite untenable that each individual of the multitude of plants in a forest of *Dendrocalamus strictus*, one thousand square miles in area in India, or of *Chusquea abietifolia* extending over a region ten miles in length in the mountains of Jamaica, should simultaneously exhaust the supply of food in the soil where they are growing.

INJURY AS A CAUSE OF THE FLOWERING OF BAMBOOS

Several interesting cases have been reported which support the theory that injury may cause anthesis in bamboos. While injury has no bearing on our problem of the natural cause of gregarious flowering in plants, yet it is worthy of consideration, since it is a probable stimulus which apparently arouses some bamboos to sexual activity.

Gamble states that single clumps of *Bambusa Tulda*, "if badly treated by over cutting or partly uprooted, will afterwards produce flowers without any general flowering" (5, p. 31).

Another instance of the flowering of bamboos being caused by injury is reported by Merrill from the Philippines. In an extensive bamboo forest of *Schizostyrium* one single culm was seen in flower. This culm had been cut by a bolo (machete). The culm was about two thirds severed and in full flower.

Knowledge of these two instances reported from India and the Philippines caused me immediately to suspect that two injured clumps of *Bambusa arundinacea* which I noticed in flower in the Buitenzorg Gardens had also flowered because of the injury received. In each clump several culms were in profuse flower, and these culms were broken off about midway of their length, while all those culms which were not in flower were healthy, uninjured shoots. It seemed possible, therefore, that the broken culms had flowered as a consequence of injury. On second thought it was evident that there was no way of determining without previous data whether the culms had flowered as a result of injury or whether they had broken as a result of flowering. The culms of *Bambusa arundinacea* die after flowering. A dead culm is much less resistant than is a live one to strain from wind, which may be very great on a culm forty to sixty feet in height. In order to ascertain which event, the flowering or the breaking, had preceded the other, I had several culms cut in a large and healthy clump of *Bambusa arundinacea*. These culms when observed one year after cutting had not flowered. The injured culms above mentioned had in all probability broken as a result of flowering and dying and consequent weakening of the culms.

My experiment in cutting these culms was merely to test the effect of injury on this particular species. I subsequently obtained data from India far more convincing. The Chief Conservator of Forests of the Madras Presidency, India, has kindly informed me that in the bamboo-forest areas, many of which are twenty square miles and more in extent, with *Dendrocalamus strictus* and *Bambusa arundinacea* as the predominating growth, the bamboo culms are worked on a rotation of three to four years.

The periodical cutting over and clearing of the individual clumps has had no known effect on the periodicity of flowering.

As for other species of bamboo, there is evidence galore showing how little injury affects the continued vegetational growth of the plants. The most common method of raising bamboos is by cuttings, and so far as I am aware all species lend themselves satisfactorily to this method. The little slender bamboo *Bambusa nana* is commonly used as a hedge plant and is therefore subjected to frequent cutting without any apparent effect on flowering.

Another form of injury which is said to produce anthesis in bamboos is burning. From the Philippines comes the report that, in a clump of *Dendrocalamus* (species not given) which had been severely injured by fire, the few uninjured or but slightly injured culms had produced flowers. The case was of especial interest because of an observation made by the writer in Jamaica. Fully ninety-eight percent of the plants of *Chusquea abietifolia* seen in the mountains of Jamaica had flowered and died in 1919. Two small patches, however, were found which contained green, healthy plants, and one of these patches had recently been burnt over. The charred stubble was still evident. The parent plants had been burnt to the ground before their life cycle was complete, and the living rootstocks had sent up new shoots which were continuing the growth of the plants and thus carrying on the vegetative portion of the life cycle beyond the normal limit. Burning here not only did not cause flowering, but had, on the contrary, apparently prevented it.

The most convincing example of bamboos flowering as a result of injury that I know of is the report of Branthwaite. He tells of the flowering of three clumps of *Dendrocalamus strictus*. The flowers were borne on short stems which had their origin just below the surface of the ground from the base of culms which had been cut for a clearing on which a hut was built (2, p. 233).

While the sum total of evidence is decidedly against the fact that flowering of bamboos can be induced by injury, the reports of Branthwaite, Merrill, and Gamble suggest that injury may at least sometimes in certain species of bamboos produce anthesis.

THE GREGARIOUS FLOWERING OF THE ORCHID *Dendrobium crumenatum*

Gregarious flowering is characteristic not only of bamboos (and to a limited extent of the talipot palm), but also of the orchid *Dendrobium crumenatum*.

Wherever a number of individuals of the orchid *Dendrobium crumenatum* occur within the same general locality, the plants flower simultaneously. The blossoms of every plant burst forth on the morning and wither in the evening of the same day.

Among the specimens of *Dendrobium crumenatum* in the Buitenzorg Gardens in Java there are plants collected from nearly all parts of the Dutch East Indies, from Riouw (near Singapore), from Sumatra, Java, Borneo, Celebes, and Ambon (a small island at the eastern end of the archipelago). These plants, after being brought to Buitenzorg, all flowered on the same day, if they flowered at all. Yet in their native habitat the flowering periods of the plants do not at all coincide. Thus, orchids growing in the virgin mountain forests flower on different days from those in the lowlands. Plants growing at two stations but three kilometers apart may differ in their times of flowering by one or two days. But wherever their original home and whatever the date of flowering there, the plants, when assembled in one locality, flower simultaneously with each other and with the plants which have grown in that locality from youth. There is no other explanation here but that some external factor determines the *exact* time of flowering. The interesting question arises, What is the controlling external factor?

Burkhill, from data obtained in the Straits Settlements, comes to the conclusion that "climatic conditions some eight days in advance of the flowering are a controlling factor" in the gregarious flowering of *Dendrobium crumenatum* (3, p. 405).

The writer has recently (21) published data from Buitenzorg which support the conclusion of Burkhill. If the flowering dates of the orchid are compared, in a table, with the daily precipitation data preceding all the flowering dates, it will be seen that in the majority of instances the rainfall on the eighth day preceding each day of gregarious flowering is unusually heavy. Especially evident does this become when the totals of the precipitation figures for the respective series of days are compared. The total rainfall occurring on the eighth day previous to all the flowering dates is five ninths greater than that of the next highest. The data strongly support the possibility that heavy rainfall eight days in advance of flowering is the cause of simultaneous flowering of the plants. But several striking exceptions occur which force one to conclude that the stimulating factor which arouses the resting flower buds to further activity is not rainfall but some other as yet unknown factor (possibly temperature) commonly associated with heavy rainfall.

At first thought, the gregarious flowering of *Dendrobium crumenatum* is

conclusive evidence that simultaneity of flowering is at least in some plants determined by an external environmental factor. Two striking differences between the gregarious flowering of *Dendrobium crumenatum* and that of bamboos tend further to support this belief. In the bamboos flowering is rhythmic. In the orchid the periods between flowering dates vary from a few days to several months. There is no rhythmic periodicity here.

The second striking difference between the gregarious flowering of orchids and that of bamboos is that in the latter case all the individuals of a bamboo forest are of the same age, while among an assemblage of orchids the individuals may be of quite different ages. Without further consideration one would be inclined to regard some external stimulus as the cause of the irregular gregarious flowering in the pigeon orchid.

The writer has shown, in the article referred to (21), that *simultaneity* of flowering in *Dendrobium crumenatum* rests not upon a climatic but upon a heritable factor, namely, the innate disposition of the plants to develop all their flower buds to the same degree of advancement, at which point growth ceases.⁶ The climatic factor arouses the buds—which are all of the same age and which, therefore, all require the same length of time (eight days) to complete development—to further activity, and thus determines merely the *exact time* of flowering and not the simultaneity of it.

CONCLUSION

As biological science progresses, many vital phenomena, which in the past have been regarded as resident wholly within the organism and in no way determined by the external environment, are one by one shown to be in many instances materially influenced, and in some instances directly determined, by environmental factors. While it must be admitted that one cannot altogether dissociate an organism from its environment, yet this hardly precludes one from regarding some vital phenomena as strictly innate. The origin of variations and mutations forces one, it seems to me, to admit the existence of at least a certain amount of independence of function of the germ plasm from its environment.

The opposition of some biologists to the belief in a hereditary disposition of plants which is responsible for periodicity (in growth, reproduction, etc.) is apparently based on a fear of giving support to any hypothesis which would attribute to a plant self-regulation and would tend to dissociate the plant from its environment. But there is nothing mysterious in periodicity any more than in the radio-activity of certain metals or in the chemical reaction which takes place in a test tube regardless of the surrounding conditions. The causes are merely internal instead of external.

Of the many vital phenomena which are rhythmic in plants—leaf production, leaf fall, cambium activity, reproduction, the synthesis and

⁶ For a complete discussion of the possible mechanism involved see the article referred to (21).

solution of starch, etc.—some are undoubtedly susceptible to environmental factors. Furthermore, the same phenomenon varies in different plants in the readiness with which it can be influenced by surrounding conditions. Thus, the winter's rest in some plants is easily altered, in others it can not by any known artificial means be broken. Klebs first clearly showed experimentally that periodicity in plants can be disturbed. In this lies his contribution to biology. But when Klebs would have us believe that *all* the activities of plants are to some extent determined by the outer world, we question his right to do this in the face of his own experiments since he found certain plants which could not be aroused from their winter's rest (15).

But even in those cases in which the normal periodicity can be disturbed, the question arises whether or not the mere ability to alter the normal rhythm of growth by changing the external environment is an indication that this periodicity is actually the direct result of an environmental rather than of a germinal factor. There are some striking instances in which the normal alternation of growth and rest is upset but the plant suffers in consequence. Disturbing the usual growth rhythm results in weakening the plant's vitality. An excellent illustration of this exists at Tjibodas, Java, where there is a small apple tree growing in the acclimatization garden in the mountains. This temperate-zone tree has been growing in the tropics for some twelve years or more, in a climate which has no pronounced seasonal change. Its normal periodicity of growth and rest has been disturbed but *not done away with*. The tree is undersized (four feet high) and has never borne fruit. It stands there an unhappy specimen, with, when I saw it, one branch in full foliage, another without any leaves at all, and still another with well-developed buds. It seems to be having a sad time trying to exist in a seasonless climate with an inherent periodicity of growth and rest manifesting itself at different times of the year on different branches. The periodicity is there. The seasonal cycle of temperate regions would have determined *when* the rest and growth periods should occur. In a tropical climate this seasonal guidance is lacking and the normal rhythm of growth and rest is disarranged, but the inherent periodicity is still evident.

The fact that the winter's rest in plants can in many cases be disturbed has led other workers than Klebs to come to rather far-reaching conclusions. Thus Howard, as a result of some very extensive work on the treatment of dormant woody plants for forcing them into growth, concludes that "all of these forms of rest are caused by unfavorable external conditions" (9, p. 5).

Just what the unfavorable conditions are which cause all kapok trees (*Ceiba pentandra*) *simultaneously* to become completely defoliated each year at Buitenzorg, where there is no pronounced seasonal change, it is difficult to see. Even more striking is the case of *Ceiba* (*C. occidentalis* ?), the silk-cotton tree) in Jamaica which annually loses all its leaves, but not simul-

taneously with other silk-cotton trees. Why do the "unfavorable external conditions" which cause one silk-cotton tree in Jamaica to rest from leaf production not likewise cause another silk-cotton tree standing near by to rest also?

Howard's further deduction, that "a plant readily adapts itself to the new demands and the rest becomes a habit," is perhaps applicable to some plants, but not to all. The tropical palm has not yet adapted itself to a temperate climate and acquired the habit of resting in the winter; nor has the temperate-zone apple tree at Tjibodas very successfully adapted itself to a tropical climate after twelve years or more of existence there, even though the climate at Tjibodas is not unlike a temperate summer as regards moisture, temperature, and light.

One fundamental objection to the belief in a heritable periodicity in bamboos has been raised by several writers. It is pointed out as a remarkable fact that "not only mature clumps but quite slender seedlings" (11, p. 126), "even the buds which have just appeared out of the ground" all blossom at the same time (19, p. 6). This brings us to the consideration of a rather theoretical question, What constitutes age?

That the parent culms in a large bamboo forest of *Dendrocalamus* or *Chusquea* are all of the same age is self-evident. They all sprang from seed sown at the same time, *i.e.*, at the time of the simultaneous death of the individuals of the previous forest. As for the "young" shoots, their age is, from one viewpoint, the same as that of their parents since they arose from the same rootstock. Meristematic cells, in the cambium ring, for example, remain perpetually young, though in years they are older than most of the cells which make up the tree. Old cells become young when lateral shoots are formed from old wood in trees, or when in lower animals limbs are regenerated. If we grant that the morphology and function of cells is dependent on their location in the plant, that is, that there is no such thing as specificity of cells, then all the cells of a bamboo clump, in "young" shoots as well as in "old" culms, are potentially the same; therefore, all are alike affected by age. Consequently, the determiner present in the germ plasm of the "old" culms which causes them to reach sexual maturity at a definite time is likewise present in the "young" culms which arise from a common rootstock.

How this innate sexual periodicity of some bamboos came into existence it is impossible to say. Either it must have been established in the past as an acquired habit, or it must be purely the expression of the original physical and chemical make-up of the germ plasm. The nicety with which the life cycle of annuals and the growth rhythm of perennials fit in with the seasonal changes of temperate regions leads one to believe that these periodic vital phenomena have been induced through the ages by climatic conditions, with the result that the periodicity has become innate, the habit being more firmly established in some plants than in others. The same

may be true of bamboos of long life cycle, although in this case the climatic factor is apparently no longer active.

The belief in a germinal factor as the cause of gregarious flowering in bamboos does not imply that this heritable determiner is past being influenced by the external environment. The finding of green specimens of *Chusquea abietifolia* in the mountains of Jamaica when fully ninety-eight percent of the total number of plants were dead suggests that the usual periodicity of this bamboo has in some individuals become altered. As was pointed out in the introduction of this article, it is the task of the biologist to ascertain to what extent this or that character is susceptible to external influence, i.e., to ascertain the degree of fixity of the innate factor. If it is found that a vital process cannot be altered, then we must admit either that it is too firmly established in the germ plasm to be disturbed, or else that we have not found the requisite environmental factor. This latter assumption is made by Klebs.

It is impossible to deny the assumption of Klebs that where we are unable to find the controlling environmental factors we have simply failed to search far enough; yet, until the exact combination of external stimuli is found, the theory that gregarious flowering is determined by a germinal factor stands without disproof.⁷

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⁷After this article was written there appeared an account by Wieland (Amer. Jour. Bot. 8: 218-230. 1921) of monocarpy in the cycadeoids. There is evidence that at least two species of fossil cycads flowered but once in a lifetime. Four other species show a tendency toward monocarpy. The most convincing record is that of a specimen of *Cycadeoidea Dartoni*, the armor of which is packed with hundreds of mature cones. Ample sections of this specimen show no trace of a succeeding foliar crown; although in the very different species, *C. ingens*, a fine crown of young fronds surmounts a scattered growth of flower buds. It is, therefore, reasonably evident that *Cycadeoidea Dartoni* was monocarpic.

It is most interesting to have so authentic a record of monocarpy among the gymnosperms of past geologic ages.

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EXPLANATION OF PLATE XII

FIG. 1. The avenue of talipot palms, *Corypha umbraculifera*, in the Peradeniya Gardens, Ceylon, in December, 1920. Seven of the sixteen palms forming the avenue flowered in June, 1918. One of the palms which had flowered stands in the front on the right side of the picture. The fruit has fallen, leaving only the bare stalks of the inflorescence.

FIG. 2. Another *Corypha* in the Peradeniya Gardens which flowered some years before those shown in figure 1, and which, unlike those palms, received for at least four years previous to the time of flowering an annual rainfall above the average normal. This picture gives some idea of the luxuriance of the inflorescence. (The photograph is published through the courtesy of Plâté, Ltd., Ceylon.)

FIG. 3. A telephotograph of the crown of a *Corypha umbraculifera*, after fruiting, in the Buitenzorg Gardens. This talipot palm had never experienced a drought; indeed, it was drenched in rain nearly every afternoon of its life, while those palms shown in figures 1 and 2 were annually subjected to a dry season which frequently assumed the proportions of a drought. Some few of the leaves of the former crown of foliage are still to be seen clinging to the trunk. The palm is dead, or nearly so.

FIG. 4. A small clump of *Dendrocalamus giganteus* in full flower in the Buitenzorg Gardens. Long pendent inflorescences are abundant, while but few leaves remain on the now nearly dead culms. These bamboo shoots were taken from an old clump of *D. giganteus* which was beginning to flower. The "young" transplanted culms flowered soon after the parent plant.



SILVER: CAUSES OF GREGARIOUS FLOWERING